BASE STRENGTH-REACTIVITY EFFECTS IN POLYETHYLENIMINE ESTEROLYSIS REACTIONS

BY

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A DISSERTATION PRESENTED TO THE GRADUATE COUNCIL OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

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TO MY WIFE

AND

DAUGHTER

JOE ANN AND SPRING

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Major Department: Chemistry

The esterolysis of p-nitrophenyl acetate (PNP) and p-nitrophenyl caproate (PNPC) by polyethylenimine (PEI) derivatives was studied as a function of pH. The reactivity of partly dodecylated PEI containing primary amines (PEI-D-NH_2-HCl), imidazole (PEI-D-Im-HCl), pyridine (PEI-D-Pyr-HCl), and 2-aminopyridine (PEI-D-APyr-HCl) was found to be pH dependent without exception. The PEI-D-NH_2-HCl system was found to react with PNPA predominantly by nucleophilic attack of polymeric primary amine. Potentiometric titration showed the $pK_{a,app}$ or PEI-D-NH_2-HCl also to be dependent. A Bronsted-like plot of log k for polymeric free primary amine against $pK_{a,app}$ for the esterolysis of PNPA with PEI-D-NH_2-HCl had a slope (β value) of 0.81. A Bronsted plot of a series of low molecular weight amines with PNPA taken from the literature had a slope of 0.83. The Bronsted-like plot of log k with $pK_{a,app}$, an

approach unknown prviously in polymer systems, quantitatively accounted for the pH dependence of the reaction of PEI-D-NH_2-HCl with PNPA. The reactivity of PEI-D-NH_2-HCl toward PNPA is dependent on the pK of the nucleophile as in the case of low molecular weight amine nucleophiles. The slope of the plot of log k versus $pK_{a,app}$ for the esterolysis of PNPC by PEI-D-NH_2-HCl had a slope of 1.06. This slope was dissected into a pH dependent polymeric enhancement component (E) and a pH dependent nucleophilic component. The $pK_{a,app}$ of the heterocycles in the heterocycle containing polymers was unobtainable in the pH region of interest. However, the pHdependencies were assigned to nucleophilic effects by analogy. This approach proved useful in reviewing literature reports of other polymeric esterolytic systems. Data for PNPA esterolysis by poly-4(5)vinylimidazole and for 2,4-dinitrophenyl acetate esterolysis were plotted as described above with slopes of 0.8 and 1.3, respectively.

CHAPTER I

POLYMER ESTEROLYSIS REACTIONS: DISCUSSION AND PROPOSAL

Introduction

Esterolysis by polymer systems has received considerable attention[1] since the early days of polymer research. This area of research was spurred as the mechanism of enzyme reactions became better understood. It was hoped that synthetic polymer systems might offer a method of modeling or duplicating the action of the naturally occurring polymers (enzymes). The aspects of enzyme action of greatest interest in this field are high selectivity and high efficiency. To this end synthetic polymer systems with particular binding properties and high reactivity have been sought.

One of the aspects of polymer esterolysis reactions which has received a great deal of attention is cooperativity, i.e., two or more functional groups in the same polymer molecule participating in ester cleavage. The original goal of the research to be discussed herein was to find and study possible cooperative effects in nitrogenous polymer systems. Chapter I will first discuss the mechanism of polymer esterolysis and aspects of possible bifunctional or cooperative mechanisms. Secondly, the design of a system

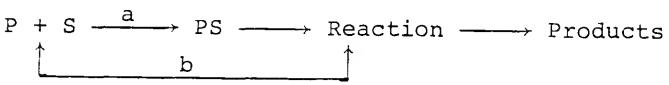
in which cooperative effects may be found will be discussed. The remainder of this dissertation is a discussion and interpretation of the results of the study and the impact of this interpretation on the field of polymer esterolysis.

Discussion

Mechanism of Polymer Esterolysis Reactions

One of the features present in polymer reactions that is generally absent in monomer reactions is the possibility of binding preassociation. A simplified scheme for such a reaction is shown in Equation I-1.

Equation I-1



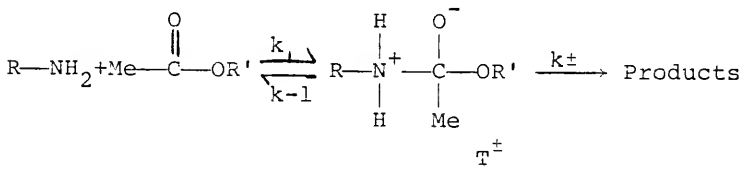
The substrate may (path a) or may not (path b) bind, depending on the structure of both the polymer, P, and the substrate, S. Binding can greatly increase reaction rates by increasing the concentration of substrate in the presence of the polymer. There are at least two modes of binding, hydrophobic and electrostatic, for synthetic polymer systems. Hydrophobic binding is the mole of interest here; it requires polymers and substrates of hydrophobic nature. Such substrates frequently contain long aliphatic chains which are usually located on the carboxyl end of the ester. The electrostatic effects of concern are those produced by a partly ionized polymer and their consequence on hydrophobic binding and nucleophilicity. These effects are quite

important, but until now they have been impossible to quantitate.

In esterolytic reactions of polymers, the binding can certainly increase the reaction rate, but the cleavage portion of the esterolysis mechanism is considered to be independent of binding.[1f] That is to say that the ester is cleaved by the polymer in the same way, whether it is bound to the polymer in a previous step or not (neglecting buffer reactions, etc.). This is a reasonable supposition in that nonspecific hydrophobic binding should not result in any significant change in the details of the reaction mechanism. This assumption, which is usually not stated, is very important in understanding polymer esterolysis reactions as it allows comparison to nonpolymeric reactions.

The specific types of esterolysis reactions of interest to this study are those of nucleophilic amines with p-nitrophenyl esters in aqueous media. Satterthwait and Jencks[2] have provided the mechanism shown in Equation I-2 for esterolysis reactions which are first order both in p-nitrophenyl ester and nonpolymeric amine nucleophile. The tetrahedral intermediate $T^{\pm}$ is formed in a rapid and reversible step. The rate determining step is the breakdown

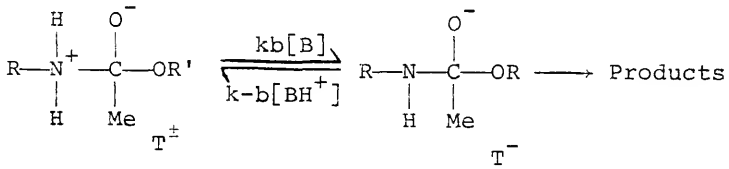
Equation I-2



of $T^{\pm}$. That is, $T^{\pm}$ is partitioned between starting materials (k-1) and products. The partitioning is governed by the relative strengths of the C-N and C-OR bonds. The bond strengths can be related to the pK_a's of the two possible leaving groups.[3] The greater the pK_a of the nucleophile, the greater the percentage of tetrahedral intermediates proceeding on to products. It is clear then that the pK_a of the nucleophilic amine is a very important measure of its reactivity in aminolysis reactions. The correlation of reaction rate with pK_a, known as the Bronsted relation, is very important to the arguments put forth later and will be discussed in more detail in Chapter II.

The breakdown of the tetrahedral intermediate may be catalyzed by general acid or general base mechanisms. The mechanism of general base catalyzed breakdown of the tetrahedral intermediate $T^{\pm}$ is shown in Equation I-3. This pathway transforms the protonated amine group into a nonprotonated amine. The amine pK_a of importance for the anionic

Equation I-3



intermediate, T^{-}, is the conversion of the amine to the amide anion. This pK_a is very much greater than that of the alcohol. The intermediate T^{-} is thus prevented from breaking down to starting materials. This general base catalyzed

pathway promotes favorable partitioning of $T^{\pm}$ toward products. The third order pathway contributes more in cases where the amine pK_a is significantly lower than that of the alcohol moiety. The general acid pathway generates the T^- intermediate via a more intricate set of proton transfers. However this pathway includes proton transfers, which for reactions of moderately basic amines, are thermodynamically unfavorable for p-nitrophenyl ester reactions[3] (proton donation from a weak acid to a weaker base). The general acid pathway is of higher energy than the general base and the uncatalyzed pathways.[4] Therefore, general acid catalyzed reactions will not be considered further.

The above discussion was largely directed at primary and secondary amines; however, it also applies to nucleophilic tertiary amines.[3] The heterocyclic tertiary amines, pyridine and imidazole, are of particular interest. Most pyridines are incapable of losing a proton in the $T^{\pm}$ intermediate ,and the T^- intermediate is therefore not accessible (Equation I-3). Nucleophilic reactions of pyridines with p-nitrophenyl esters must pass through the second order

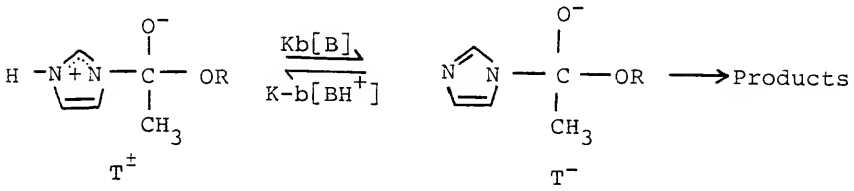
Pyridine

Imidazole

uncatalyzed pathway. Imidazole on the other hand is not restrained from access to the T^- intermediate. The imidazole may lose a proton from the $T^{\pm}$ intermediate to form T^-. This pathway is analogous to the pathway proposed by Satterthwait

and Jencks[3] for the general base catalyzed aminolysis of esters by primary and secondary amines. Satterthwait and

Equation I-4



Jencks did not discuss the scheme in Equation I-4. However, this mechanism has been proposed for nonaqueous systems.[5] This discussion is not meant to imply that there is a general base catalyzed imidazole acylation reaction with p-nitrophenyl esters in aqueous systems, but for the purposes of this discussion any general base catalysis will be assumed to follow this mechanism.

The general base catalyzed reaction discussed above favors partitioning of the intermediate $T^{\pm}$ to products by formation of T^{-} (Equation I-3 or I-4). If the pK_a of the amine nucleophile is not greatly larger than the alcohol leaving group then, the second order uncatalyzed pathway (Equation I-2) would not partition as efficiently as the third order general base catalyzed route. In terms of activation parameters, the third order pathway for phenyl esters in general is lower in activation enthalpy than the second order pathway. However, third order reactions are of lower activation entropy than second order reactions. The decrease in activation entropy tends to overcome the decrease

in activation enthalpy. As a result the second order pathway is frequently of lower energy than the third order pathway.[4]

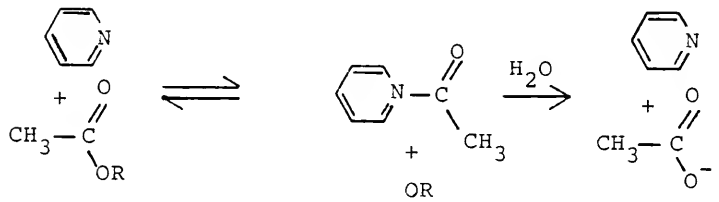
It is clear from the above discussion that if the problem of activation entropy reduction could be resolved, esterolysis reaction rates could be enhanced via the general base catalyzed route (Equation I-3 or I-4) in some cases. If the general base and nucleophile were part of the same molecule, then the general base pathway would no longer be third order. The entropy of activation reduction would be resolved largely. Addition of more general base species to the nucleophile containing molecule should reduce the free energy of activation for the general base reaction further. A polymer containing nucleophiles and species capable of serving the general base function should lower the free energy of activation for the general base catalyzed reaction below that of the uncatalyzed reaction. Such a bifunctional or cooperative interaction should enhance the reaction rate. The extent of the enhancement would depend on the nucleophile-ester pair studied. As long as the formation of the intermediate $T^{\pm}$ (Equation I-2) is rapid and reversible, the greater the pK_a of the alcohol leaving group relative to the pK_a of the nucleophile, the more effective is the enhancement. Conversely, if the pK_a of the alcohol leaving group is low relative to the pK_a of the nucleophile, then the partitioning of the intermediate $T^{\pm}$ would be very efficient. In the latter case the general base catalyzed reaction would

not be significantly lower, and no enhancement should be observed.

Polymer Catalyzed Hydrolysis

The initially formed products of the ester aminolysis reactions are amides. However, in some cases these reactions can be viewed as the first step of ester hydrolysis reactions. An amine may serve as a nucleophilic catalyst if the initially formed amide is hydrolyzed at a reasonable rate (Equation I-5). In actual practice nucleophilic tertiary amines, pyridines or imidazoles are usually used for these reactions. These tertiary amines have a reasonable acylation rate as well as deacylation rate.[1] However, the first step of the reaction, the aminolysis step, is the point of concern here. The actual fate of the ester after release of alcohol moiety is of little consequence. Thus the structure of the amine nucleophile relative to its deacylation is also of little consequence. Therefore, the aminolysis reaction by primary amines (Chapter II) will be used as a model for

Equation I-5



aminolysis (hydrolysis) reactions of tertiary amines (Chapter III).

Overberger et al.[6] have published data that they interpret as being consistent with a bifunctional mechanism. They studied the hydrolysis of p-nitrophenyl acetate catalyzed by poly-4(5)-vinylimidazole and imidazole as a function of the fraction of unprotonated imidazole units[6] (Figure I-1a). They found that although the imidazole esterolysis rate increases linearly with α, the polymer rate increases nonlinearly. They concluded that neighboring nonprotonated imidazoles interact cooperatively, enhancing the rate of reaction. At low values of α few nonprotonated imidazoles are neighbors, and the reaction presumably proceeds through a noncooperative pathway. As the value of α increases the cooperative interaction becomes more and more the predominant pathway, and the reaction rate increases accordingly. The mechanism of Jencks and Satterthwait (Equation I-2, I-4) although different from those proposed by Overberger et al. (Figure I-1b) is also consistent with their interpretation. In the terms discussed previously, the reaction would proceed via the uncatalyzed (Equation I-2) path as α increases.

It should be pointed out that the bifunctional interpretation of Overberger et al. has not been without criticism. Kunitake and Okahata[1b] notes that later work on poly-4(5)-vinylimidazole shows considerable conformation change, with changes in solvent polarity.[7] When the polymer is in its more compact conformation, it provides a more efficient catalysis of long chain phenyl esters. He suggests that (p 178)

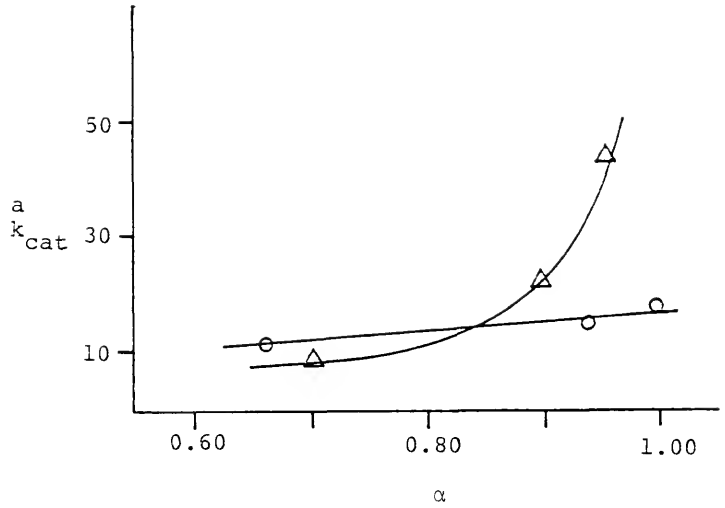


Figure I-1a. Solvolysis of PNPA catalyzed by poly-4(5)-vinylimidazole (Δ) and imidazole (O).[6]
[a] 28.5 v/v% EtOH-H_2O, u=0.02, 30°C.

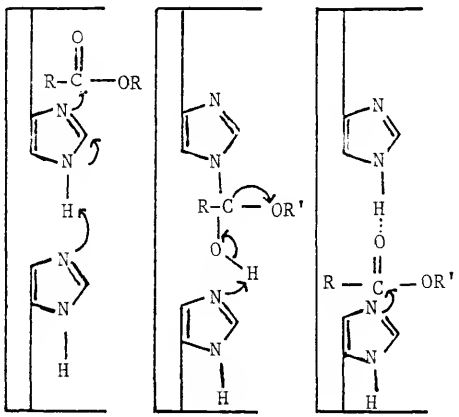


Figure I-1b. Bifunctional mechanisms proposed for poly-4(5)-vinylimidazole catalyzed hydrolysis of PNPA.[6]

"the increased catalytic efficiency observed at higher pH's may similarly be explained on a microenvironmental effect."[1b]

Summary

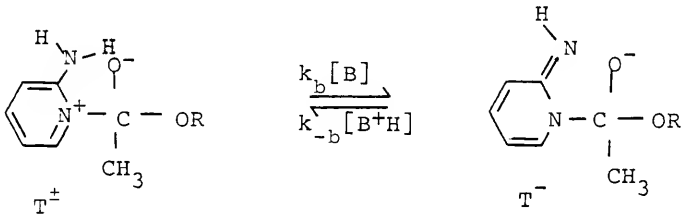
In summary, there are two p-nitrophenyl ester aminolysis reactions of concern. These are the uncatalyzed reaction (Equation I-2) and the general base catalyzed reaction (Equation I-3, I-4). The general base catalyzed reaction would be faster than the uncatalyzed reaction (for nucleophile pK_a's not much greater than alcohol pK_a's) if it were not third order. The general base reaction can be made second order by including the general base in the same molecule as the nucleophile. This effect should be increased by surrounding the nucleophile with general base functions as might be found in a polymer system. The reaction of the appropriate nucleophile ester pair should then be enhanced in the appropriate polymer due to contribution of the general base catalyzed reaction. Therefcre it is reasonable to look for the existence of bifunctional or cooperative effects in polymeric esterolysis reactions based on the above discussion, if suitable reactants are studied. Failure to find a bifunctional effect could be due to choice of the wrong reactants. It is only possible then to attempt to choose a system in which the polymeric bifunctional reaction may be unambiguosly observed.

Proposal

Introduction

There are several variables involved in the selection of the proper system in which to look for bifunctional catalysis. First one must choose the nucleophiles. Nucleophiles both capable and incapable of bifunctional catalysis are needed in order to distinguish the two pathways. Second, the polymer must contain general bases capable of removing a proton from the $T^{\pm}$ intermediate. Third, the appropriate

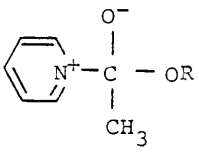
Equation I-6



ester must be chosen.

Nucleophiles

A goal of the work originally was to design a very efficient ester hydrolysis catalyst. To this end heterocyclic nucleophiles were chosen. Imidazole, due to its frequent occurrence in esterolytic enzymes, is often studied



in synthetic esterolytic polymer systems.[1] Furthermore, Overberger and Morumoto[7] claimed a bifunctional mechanism

for this nucleophile in poly-4(5)-vinylimidazole. It was decided, then, to compare imidazole to other similar heterocycles to determine if it had any special properties in esterolysis reactions. The similar structure 2-aminopyridine was also chosen. The $T^{\pm}$ intermediate formed from 2-aminopyridine is structurally capable of losing a proton (Equation I-6) just as the $T^{\pm}$ intermediate formed from imidazole (Equation I-4). The pyridine nucleophile was chosen as a nonbifunctional comparison. Unlike 2-aminopyridine and imidazole, pyridine is structurally incapable of losing a proton from $T^{\pm}$ to form T^{-}. Pyridine then cannot react via the general base pathway but only through the uncatalyzed pathway.

Polymer

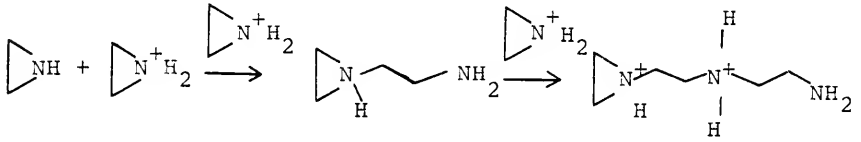
Instead of studying a homopolymer as did Overberger et al.,[6] a polymer system was chosen in which the basic sites remained constant but the nucleophiles could be varied. What was needed then was a polymer containing basic units to which the nucleophilic sites could be attached. The different nucleophiles then could be studied in the presence of a general base of constant strength.

Polyethylenimine (PEI as furnished by Dow) seemed to be the ideal system. Dow PEI 600 (Mw=40,000-60,000) is derived from cationic ring opening polymerization of aziridine (ethylenimine) as shown in Equation I-7.[8] The structure of the polymer can best be understood via a discussion of its formation. If the reaction proceeded as in Equation I-7

the polymer would consist of only secondary amines (ignoring end groups). However the polymer has been shown to contain primary, secondary and tertiary amines. This observation is readily incorporated into the mechanism by allowing the aziridinium ion to react with both primary and secondary amines. This mechanism would provide a branched polymer. Every branch produces a tertiary and a primary amino group. Thus the ratio of primary to tertiary amines is 1:1. The end groups comprising less than 1% of the total amine functions are ignored in this calculation.[8,9] The tertiary amine content has been determined by titration after exhaustive benzoylation to be 25%. Simple arithmetic reveals the primary amine content to be 25% and the secondary amine content to be 50%. Thus the ratio of primary:secondary: tertiary amines is 1:2:1.

Klotz[10] has published extensively on the properties of PEI and its derivatives. It has been shown that the primary

Equation I-7



amines of PEI are very reactive toward p-nitrophenyl esters. This is especially true for partially dodecylated and dodecanoylated derivatives reacting with apolar substrates.[11] A great deal of this enhanced reactivity is apparently due to binding. Of particular interest is a PEI 600 derivative of

which 10% of its nitrogens have been dodecylated, and 15% of its nitrogens have been alkylated with 4(5)-chloromethylimidazole.[12] This "synsyme" is reported to approach α chymotrypsim in catalytic activity. The acylation rate (excess nucleo-phile) is 2700 M^{-1} min^{-1} for the synzyme with p-nitrophenyl caproate compared to 10,000 M^{-1} min^{-1} for α chymotrypsin with p-nitrophenyl acetate.

The synzyme system of Klotz was chosen as the polymer to study. Replacement of the imidazole group with pyridine or 2-aminopyridine is readily carried out owing to the synthetic versatility of PEI. Klotz et al. have shown that the polymer may be derivatized by alkylation,[12] acylation,[12] and reductive amination.[12,13]

Ester

P-nitrophenyl acetate and caproate were chosen as esters to study.[11] The p-nitrophenyl esters are convenient for kinetic analysis. The reactions are readily followed spectrophotometrically.[14] Furthermore the great majority of polymer esterolysis studies have been carried out on p-nitrophenyl esters.[1] The caproate ester binds very strongly to the dodecylated PEI systems[11] and therefore proceeds through path a of Equation I-1. On the other hand, the acetate does not bind nearly so well[11] and must proceed largely through path b. The use of these two esters provides an insight into the binding effects of the polymer as well as being comparable to the other studies in the area.

Results

It was reasoned that possible bifunctional effects would become apparent in pH rate profiles of the polymer esterolysis reactions. With increasing pH a larger and larger fraction of the backbone nitrogens should be able to act as general bases to convert the intermediate $T^{\pm}$ into T^{-} enhancing the reaction rate. If such were the case, the reaction rate should be pH dependent. If such were not the case there should be no pH dependence unless polymer charge density has an effect on rate. Overberger and Salamone,[1e] though noting some controversy, states ". . . it appears that the varying charge density does not significantly alter the reactivity of a catalytically active polyion toward a neutral substrate" (p 218).

The pH rate profiles for the aminolysis reactions by the various polymer reactions with p-nitrophenyl acetate are shown in Figures I-2, I-3, I-4 and I-5. It is clear that the PEI-D-Pyr-HCl (pyridine system) is pH dependent. As will be pointed out in Chapter III, the pyridine in PEI-D-Pyr-HCl remains in the nonprotonated nucleophilic form throughout the pH range. Furthermore the $T^{\pm}$ intermediate formed from pyridine does not have access to T^{-}. Therefore the pH dependence for the PEI-D-Pyr-HCl system cannot be due either to ionization of the pyridine or a bifunctional mechanism. In view of Overberger's statement in this regard and its possible influence on polymer reactions, the nature of this pH dependency is very

important. The remainder of this dissertation is an effort to understand and quantitate this pH dependency.

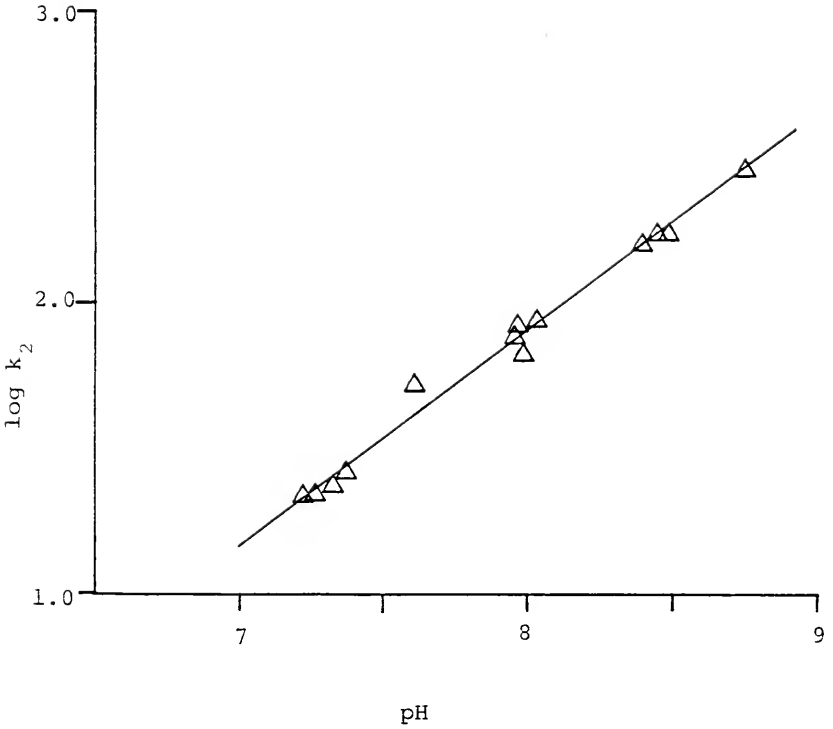


Figure I-2. Esterolysis of PNPA by PEI-D-Pyr-HCl (slope = 0.74). See Chapter IV for experimental details.

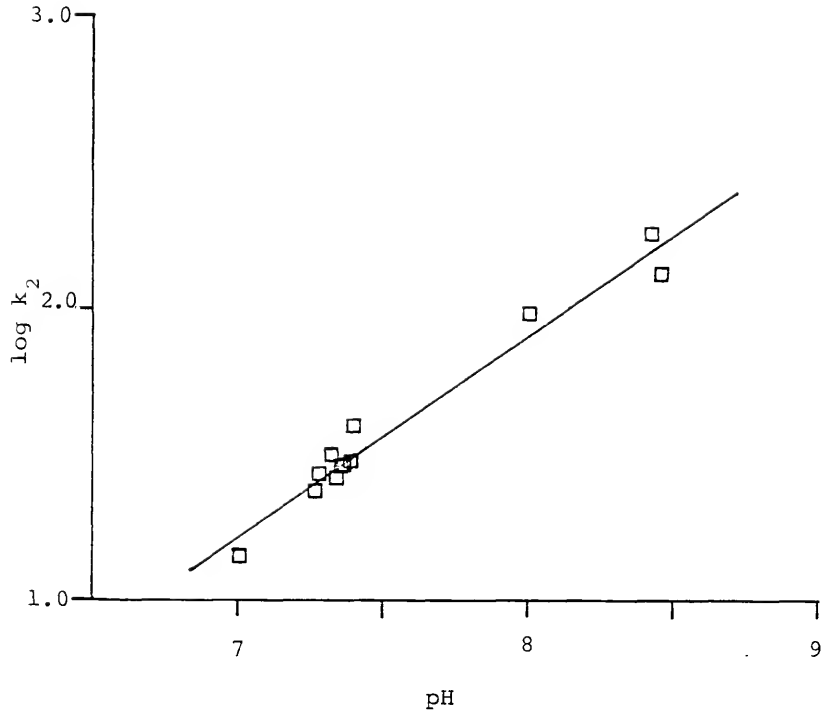


Figure I-3. Esterolysis of PNPA by PEI-D-APyr-HCl (slope = 0.69). See Chapter IV for experimental details.

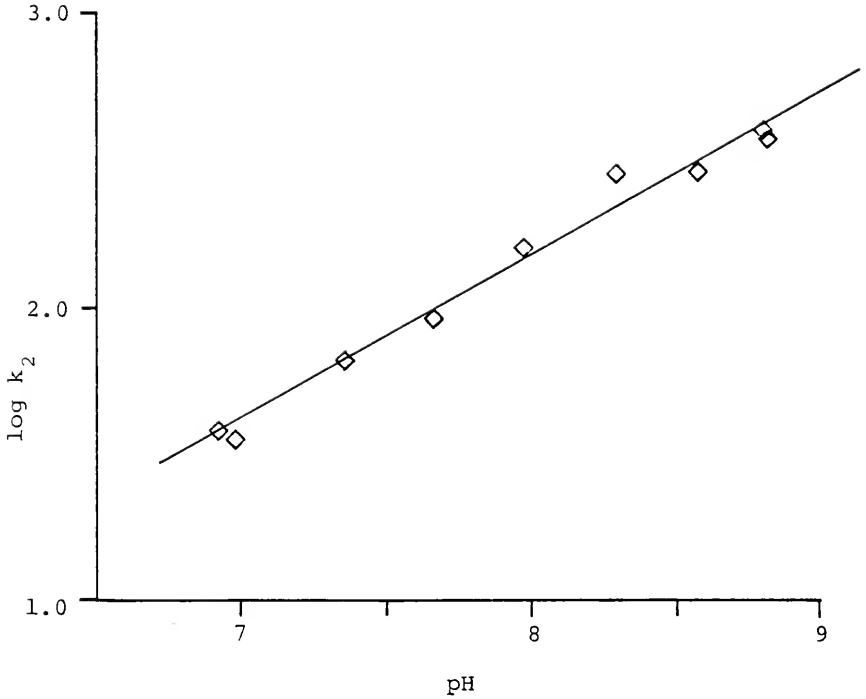


Figure I-4. Esterolysis of PNPA by PEI-D-Im-HCl (slope = 0.56). See Chapter IV for experimental details.

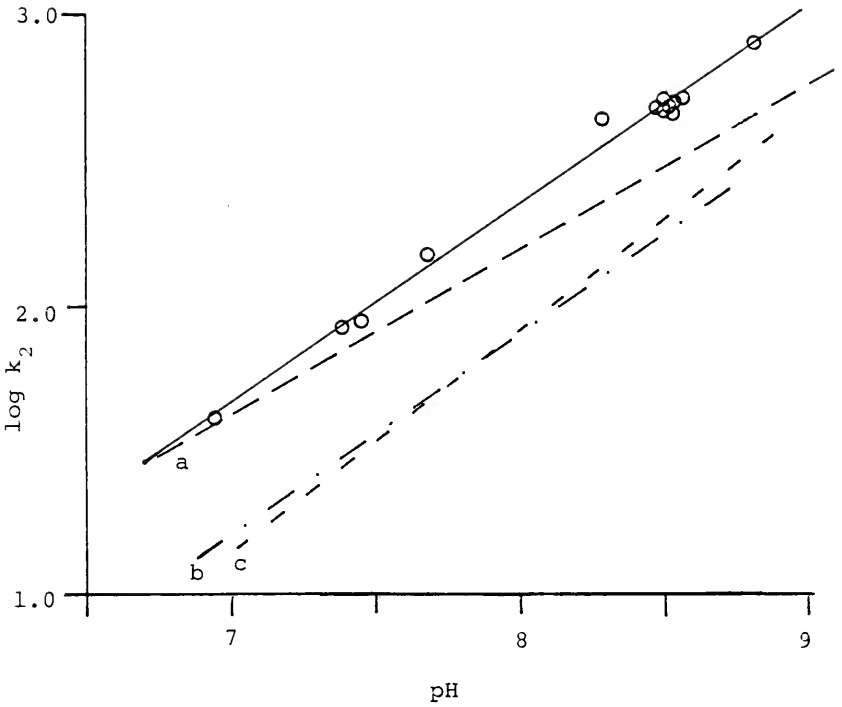


Figure I-5. Esterolysis of PNPA by PEI-D-NH_2-HCl compared to esterolysis of PEI bound heterocyclic systems. See Chapter IV for experimental details.

(a) PEI-D-Im-HCl
(b) PEI-D-Pyr-HCl
(c) PEI-D-APyr-HCl

CHAPTER II

AMINOLYSIS OF P-NITROPHENYL ESTERS BY DODECYL POLYETHYLENIMINE

Results and Discussion

Characterization

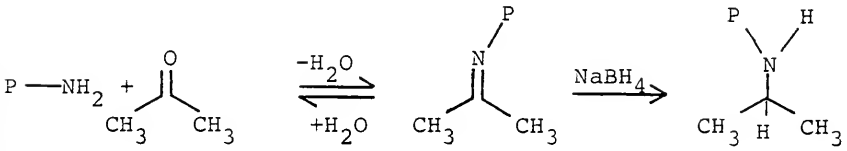
A large quantity of PEI-600 was alkylated with dodecyl iodide.[10] The PEI-D from this preparation was used to prepare all the systems studied. This precaution served to maintain a constant level of dodecylation from system to system.

The simplest system studied, PEI-D-NH_2-HCl, was the HCl salt of PEI-D. The high precision of the duplicate elemental analysis shows that PEI-D-NH_2-HCl is homogenous (this was true for all systems Table IV-1). The C/N ratic from elemental analysis can be used to calculate the extent of dodecylation as described in detail for Table IV-1. The fraction of backbone nitrogens dodecylated in PEI-D-NH_2-HCl as determined from the C/N ratio, 11%, is in good agreement with the value from NMR spectral analysis, 10% (Table IV-2).

The other polymer system to be discussed in this chapter is PEI-D-Ip-HCl. This system was prepared by exhaustive isopropylation of PEI-D with acetone and $NaBH_4$

(Equation II-1). NMR spectral analysis of the extent of

Equation II-1



isopropylation was not possible due to interference of the dodecyl group. However the extent of isopropylation can be determined from elemental analysis. The C/N ratio for PEI-D-Ip-HCl gives a value of 0.32 for the fraction of nitrogens isopropylated.

Primary amine determinations were made using trinitrobenzene sulfonate in a modification of the procedure developed by Satake et al.[15] Reportedly this method is specific for primary amines in the presence of secondary amines. Primary amine analysis of PEI-D-NH_2-HCl provides 0.20 as the fraction of primary amines, whereas analysis of PEI-D-Ip-HCl gives a value of 0.01 (Table II-1).

TABLE II-1

PRIMARY AMINE AND ISOPROPYL CONTENT OF PEI-D-NH_2-HCl AND PEI-D-Ip-HCl

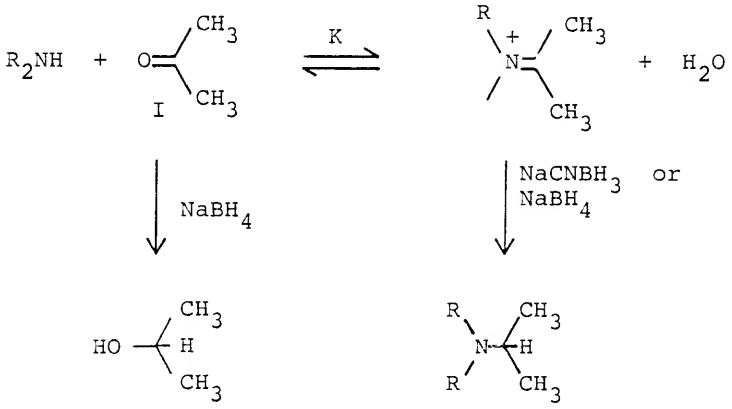
Polymer	% Primary Amines[a]	% Isopropylation[a]
PEI-D-NH_2-HCl	20	....
PEI-D-NH_2-Ip-HCl	1	32

a Values rounded to the nearest whole %; see Chapter IV for experimental details.

The above result may be surprising as it requires isopropylation of secondary amines with $NaBH_4$. Only 20% of the backbone nitrogens of PEI-D, the starting material, were primary amines. However after isopropylation 32% of the total backbone amines were isopropylated. Therefore a significant number of secondary amines must have been isopropylated.

It has been well established that unhindered ketones can be aminated with secondary amines using the less reactive $NaCNBH_3$.[16] However examples of this reaction with $NaBH_4$ are few.[17] The reason for this selectivity seems to involve the competition for hydride between the ketone(I) and the much more reactive but less abundant iminium ion (II)

Equation II-2



(Equation II-2). The $NaCNBH_3$ reagent does not reduce the ketone to a significant extent, but the more reactive $NaBH_4$ does.[16] Iminium ion reduction must occur to a greater

extent under the conditions used for preparation of PEI-D-Ip-HCl than usual. This can be explained in part by the use of a huge excess of both $NaBH_4$ and acetone. In addition the polymer may very well promote formation of the iminium ion as well. This possibility is suggested by the study of acetone H-D exchange, catalyzed by PEI, carried out by Hine.[18] They have shown that PEI (as well as certain diamines) catalyze H-D exchange, which proceeds via formation of an iminium ion, much more efficiently than monoamines. The observation of secondary amine isopropylation is important in view of the past usage of acetone/$NaBH_4$ as a method of determining primary amine content of PEI.[10]

Basicity of PEI-D-NH_2-HCl

The acid-base properties of PEI-D-NH_2-HCl were determined by potentiometric titration (see Chapter IV for experimental details). The values of α, fraction of nonprotonated polymeric amine, after each addition of titrant, were given by the concentration of added titrant divided by the concentration of polymeric amine units, CH_2CH_2N. The values of $pK_{a,app}$ at each value of α was calculated from the Henderson-Hasselbalch equation[19] (Equation II-3, see Appendix).

Equation II-3

$$pK_{a,app} = pH + \log \frac{1-\alpha}{\alpha}$$

From the plot of $pK_{a,app}$ vs α (Figure II-1) it can be seen that the basicity, $pK_{a,app}$, is dependent on the fraction of polymeric amines protonated, α. A number of factors which might well be dependent on α probably have an influence on the $pK_{a,app}$ such as conformation, ion pairing, etc. However, the important fact here is that the $pK_{a,app}$ is dependent on α and therefore pH. The dependence of the polymeric $pK_{a,app}$ on α is no surprise if short chain polyamines are used as models. The pK_a values for ethylenediamine, diethylenetriamine and triethylenetetramine[20] can be determined discretely for each stage of ionization. The values of these pK_a's are plotted against α in Figure II-1. The pK_a's of these systems follow qualitatively the same α dependence trend as the polymer. Since the polymer is not crosslinked and the $pK_{a,app}$ dependence on α is similar to the pK_a dependence on α for low molecular weight model compounds, conformations of the polymer exposing all basic sites are assumed to be in rapid equilibrium.

Kinetic Analysis

Rates of disappearance of substrate, p-nitrophenyl acetate (PNPA) or p-nitrophenyl caproate (PNPC), were monitored spectrophotometrically by observing the increase in p-nitrophenoxide absorbance. These reactions were studied under pseudo first order conditions (excess polymer) at pH's within the range 6.5-9.0. The second order rate constants (k_2 M^{-1} min^{-1}) were calculated, after subracting background rate (see Chapter IV), by division by concentration of primary amine unless otherwise indicated.[14]

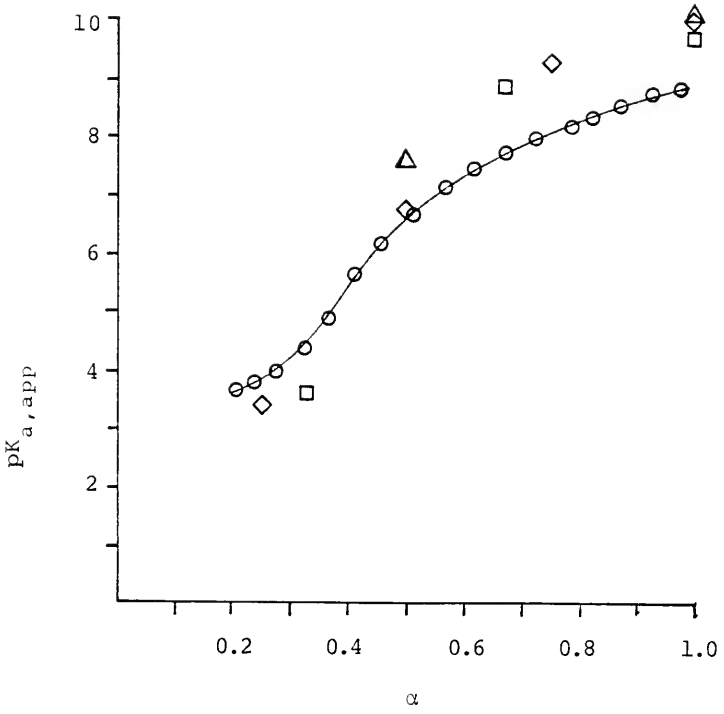
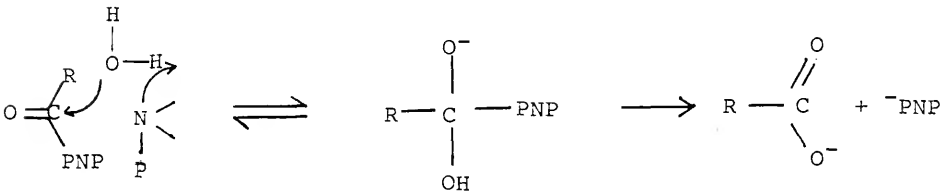


Figure II-1. Comparison of $pK_{a,app}$ dependence on α for PEI-D-NH_2-HCl with low molecular weight poly-amines.[20] (O) PEI-D-NH_2-HCl, (Δ) ethylene-diamine, (□) diethylenetriamine, (◇) triethyl-enetetramine.

Two different polymeric esterolysis processes may contribute to the pH dependence of p-nitrophenoxide release, aminolysis and/or general base catalyzed hydrolysis. The general base catalyzed hydrolysis and nucleophilic or aminolysis reaction produce different products. The general base reaction generates the carboxylate ion (Equation II-4), whereas the aminolysis reaction produces the amide[21] (Equation II-5). Both reactions release the species followed kinetically, p-nitrophenoxide. The general base route was ruled out as a significant contribution to the rate.

First, the contribution of the general base component (cf Equation II-4) can be shown to be minimal. Isopropylation of PEI-D (forming PEI-D-Ip-HCl) converts virtually all the primary amines to secondary amines. Rate constants from this system should estimate an upper limit for the general base component. In order to compare the rate data for these two systems the second order rate constants (k_{2GB}) must be calculated based on the concentration of basic sites, CH_2CH_2N units (Figure II-2). In comparing the rates

Equation II-4



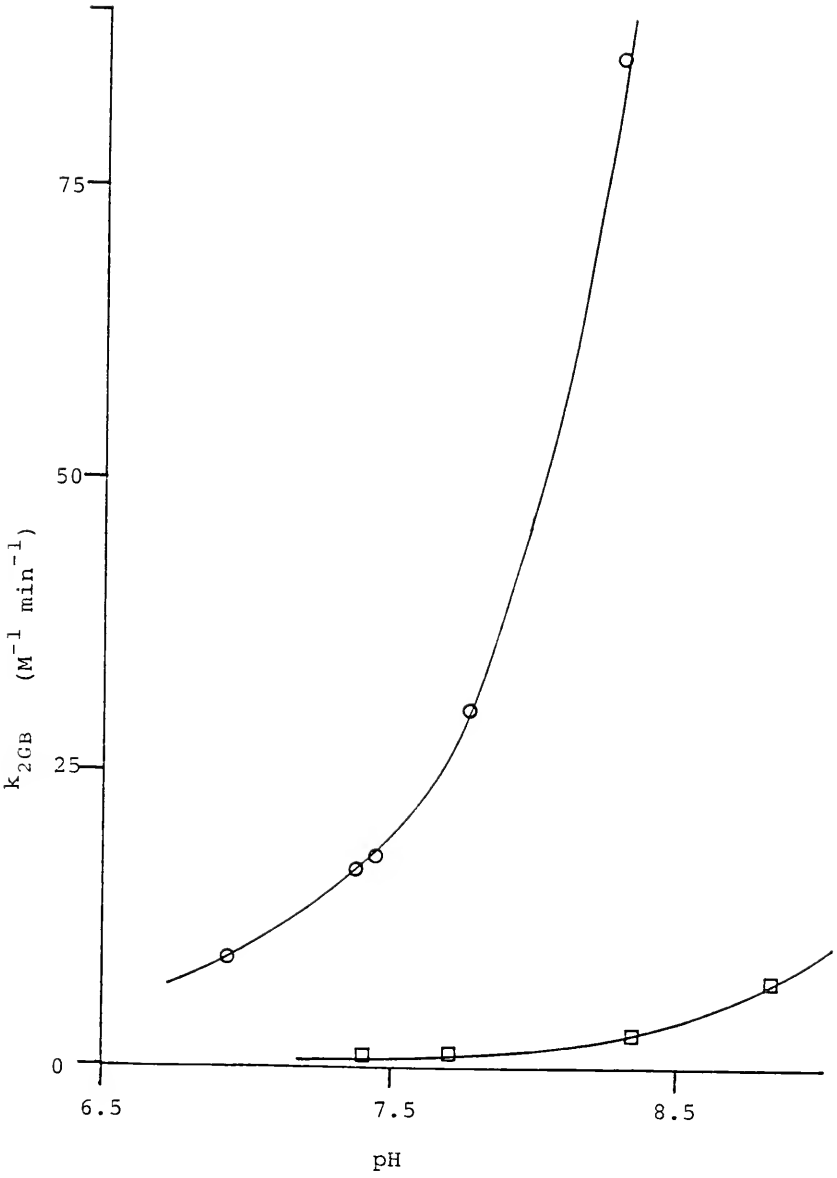
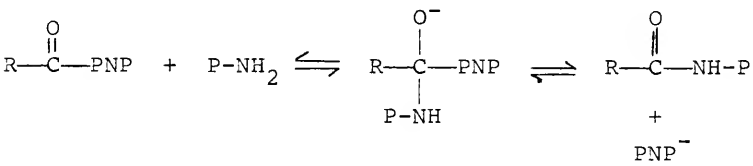


Figure II-2. Comparison of esterolysis rates for (o)PEI-D-NH_2-HCl and (□)PEI-D-Ip-HCl.

for the PNPA reaction it can be seen that the maximum contribution of the general base reaction is less than 3%.

Secondly, the reaction products can be shown to be not those of the general base reaction. The p-nitrophenoxide anion inhibits the reaction of PNPA with PEI-D-NH_2-HCl (Figure II-3). This inhibition is not due to a normal salt effect (ionic strength) as the ionic strength is 0.1, whereas the maximum p-nitrophenoxide concentration is 1.5×10^{-4}, a variation of less than 0.2%. Nor is this effect peculiar to the polyion since the same reaction with ethylenediamine hydrochloride is also depressed to a similar extent. This effect then can be compared to the common ion effect of carbonium ion chemistry. The p-nitrophenoxide ion is acting as a nucleophile on either an intermediate or product, reforming starting material. This is not possible in the case of general base hydrolysis, as neither the tetrahedral intermediate nor the product, carboxylate ion, is susceptible to nucleophilic attack (cf Equation II-4). However the result of direct attack of the amine on the ester generates the amide which is susceptible to attack by the p-nitrophenoxide ion (Equation II-5).

Equation II-5



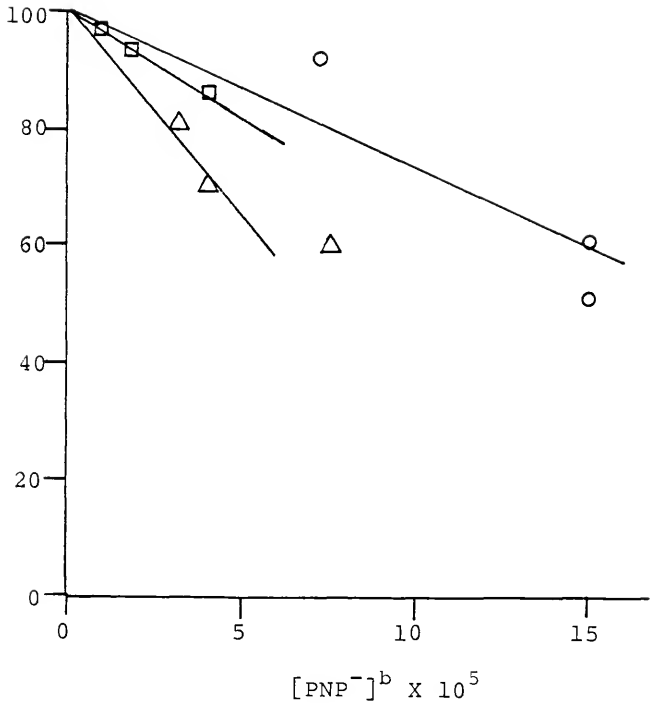


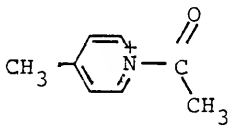
Figure II-3. Inhibition of (□)ethylenediamine, (Δ)PEI-D-NH_2-HCl (pH=7.00) and (o)PEI-D-NH_2-HCl (pH=8.82) esterolysis of PNPA by p-nitrophenol anion (PNP).

[a] % Reduction refers to the % the rate constant is reduced in the presence of PNP^- from the rate constant at very low $[PNP^-]$.

[b] The PNP^- concentration is based on p-nitrophenyl pK_a=7.14.[22]

Inhibition by p-nitrophenoxide ion has been observed by Jencks and Gilchrest in 4-methylpyridine catalyzed hydrolysis of PNPA. In this example p-nitrophenoxide attacks the reactive acetylpyridimium intermediate. However, p-nitrophenoxide attack on the much less reactive amides described here was surprising.

Based on the above results some statements can be made



Acetyl-Pyridinium Intermediate

about the mechanism of the aminolysis reaction. The reaction proceeds by nucleophilic attack of primary amine on the ester. The tetrahedral intermediate is assumed but not required. The p-nitrophenoxide anion is lost in a reversible step to form the amide. However, the question of pH dependence remains unresolved.

In small molecule systems pH dependencies can often be explained on the basis of ionization. A protonated amine cannot act as a nucleophile, and thus the rate is dependent on the fraction of free amines.[23] In the PEI-D-NH_2-HCl case correction for ionization does not remove the dependency. The pH reate profiles for PEI-D-NH_2-HCl (k_2 based on primary amine and divided by α) for both PNPA and PNPC still exhibit pH dependency after correction for ionization (Figure II-4).

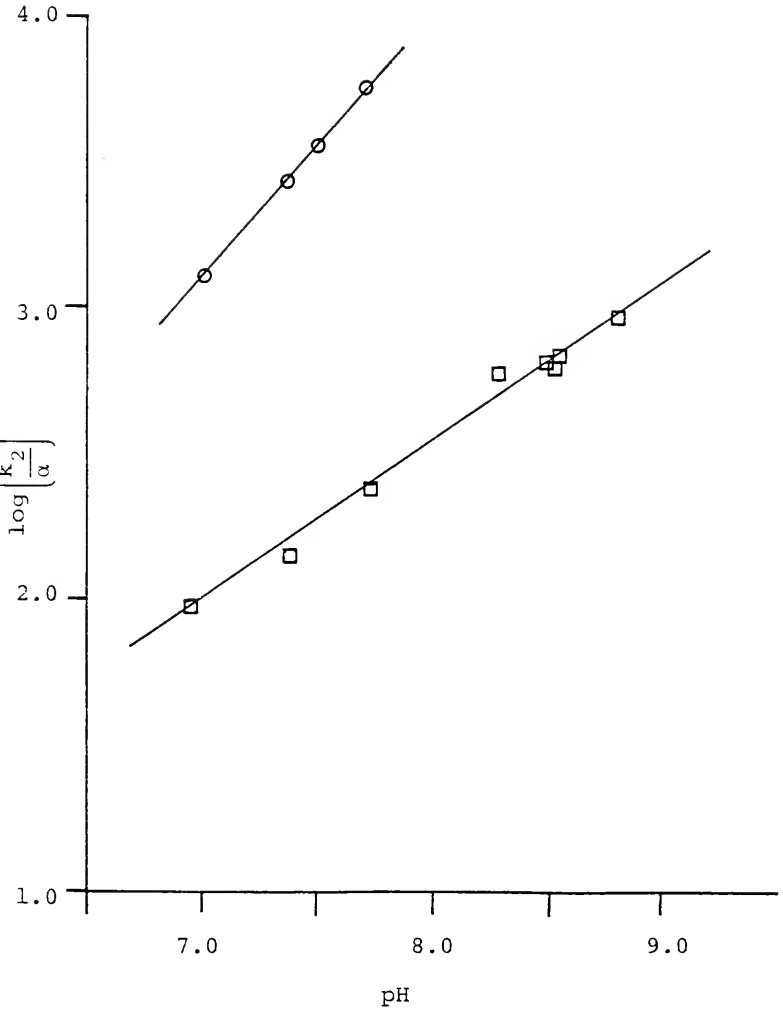


Figure II-4. Plot of $\log(k_2/\alpha)$ against pH for PEI-D-NH_2-HCl esterolysis of (o) PNPC and (□) PNPA.

The question of pH dependence in polymeric systems has been explained in several ways. One explanation involves pH dependent exclusion. As the charge density of the polymer increases hydrophobic interactions decrease, excluding the hydrophobic substrate.[24] Restated, the binding increases with increasing α thereby increasing the rate.

A second explanation relies on a bifunctional mechanism. Overberger et al.[6] have explained rate dependencies on α (which in turn is dependent on pH) in poly-4(5)-vinylimidazole reactions with PNPA as being due to interaction of adjacent imidazoles. These interactions presumably increase the reaction rate (Chapter I).

A third explanation invokes the tenet relating nucleophilicity to basicity. That is, as the pK_a (basicity) increases so does the nucleophilicity and thus the reactivity. This explanation was used by Letsinger and Saveride[25] for a dependence of rate on α. The relationship between rate and pK_a will be explored quantitatively for the PEI-D-NH_2-HCl system in the next section via the Bronsted relationship.

Bronsted Relationship

The Bronsted relationship, relating pK_a to rate constants (Equation II-6), has been used extensively in quantifying esterolysis kinetic data.[26] This relationship

Equation II-6

$$\log k/k_0 = \beta\, pK_a + C$$

can be applied to a variety of nucleophilic addition and displacement reactions. However, discussion here will be limited to ester aminolysis reactions. The values of the parameters, β and C, can be obtained by plotting the values of log k_2 (corrected for ionization) against pK_a for a series of structurally similar nucleophiles. The value of β is taken as a measure of the contribution of basicity to nucleophilicity. An example of such a plot can be seen in Figure II-5. In this case the β value for a series of primary and secondary amines reacting with PNPA is 0.83. A β value of this magnitude indicates considerable sensitivity to base strength in the reactivity of the nucleophile. The Bronsted β value is not particularly sensitive to reaction conditions. In Figure II-6 Bronsted plots for imidazoles and anilines reacting with PNPA at conditions significantly different than the amine reactions (Figure II-6) still exhibit a β value of approximately 0.8. The value of C, the vertical juxtaposition of the parallel lines, is sensitive, however, to nucleophile structure and to reaction conditions.

The dependence of the nucleophilicity of primary and secondary amines on base strength raises the question, "Can a Bronsted relationship be used to explain the pH dependence of the PEI-D-NH_2-HCl system?" Conventional Bronsted plots require data from a number of different nucleophiles since monobasic systems have one pK_a and therefore one Bronsted point, dibasic systems require two pK_a's, etc. Conversely, the $pK_{a,app}$ of the polybase PEI-D-NH_2-HCl is pH dependent (Figure II-7); therefore a well defined Bronsted plot can be

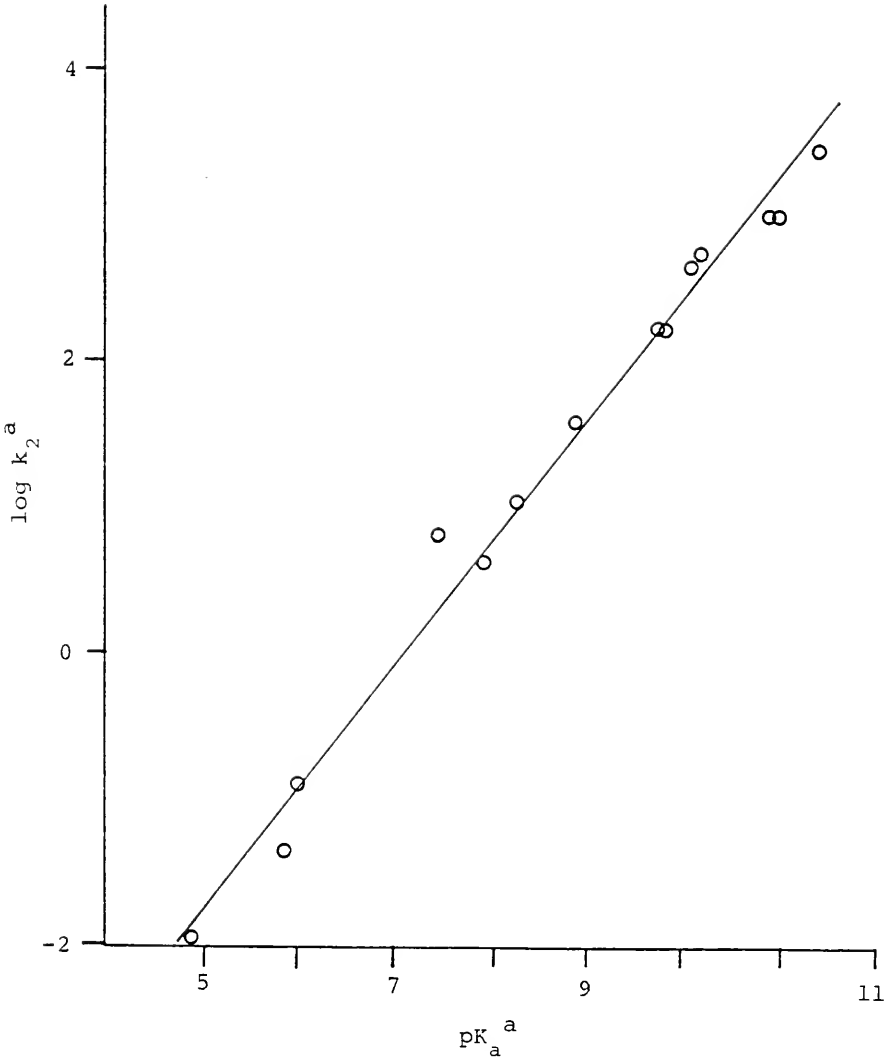


Figure II-5. Bronsted plot for the esterolysis of PNPA by simple primary and cyclic secondary amines.

[a] Values of k_2 and pK_a determined at 25°C and 1.0 M ionic strength.[22]

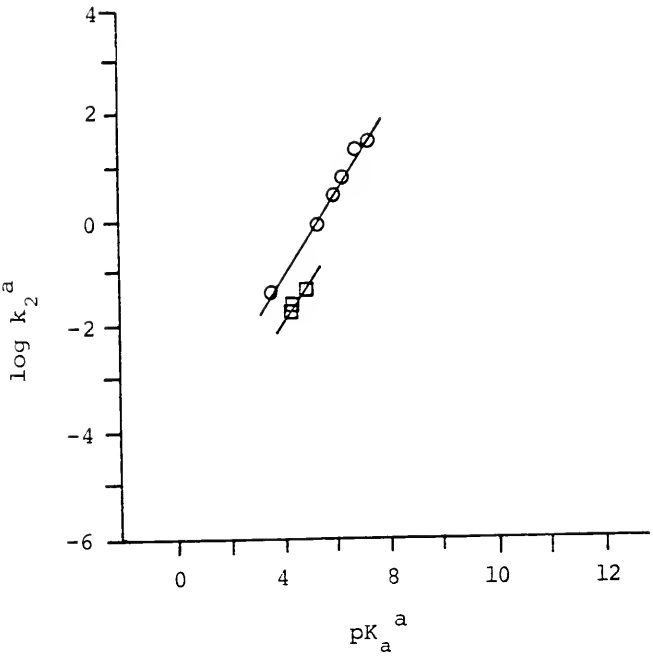


Figure II-6. Bronsted plot for the esterolysis of PNPA by (o) imidazoles and (□) anilines.

[a] Values of k_2 and pK_a determined at 30°C in in 28.5% ethanol.[27a]

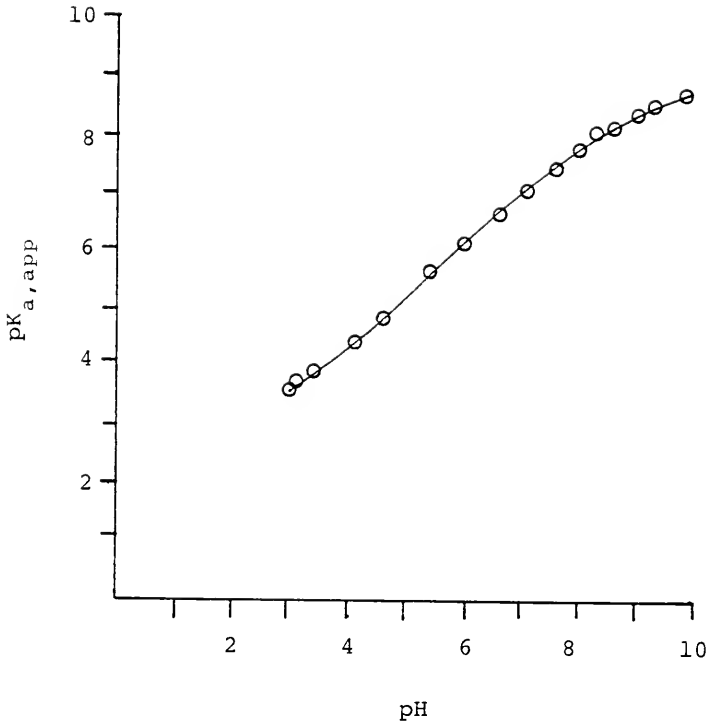


Figure II-7. Plot of $pK_{a,app}$ vs. pH for PEI-D-NH_2-HCl.

constructed from the one system. The Bronsted plots for the reactions of PEI-D-NH_2-HCl with PNPA and PNPC are shown in Figure II-8. Included on the same coordinate system is the reference plot for the simple primary and secondary amines discussed earlier. It can be seen from the values of β for PNPA with PEI-D-NH_2-HCl, 0.81, and reference, 0.83, that the difference in pK_a dependence is insignificant for the two systems. Therefore the pH dependence for the PEI-D-NH_2-HCl reaction with PNPA is completely accounted for by the dependence of nucleophilicity on base strength. However the value of β for the reaction of PEI-D-NH_2-HCl with PNPC, 1.06, is significantly different from the reference value.

The dissection of the polymeric rate can be facilitated by assuming that k_2/α can be separated into two terms, k_a and E (Equation II-7). If the term E is taken to be the rate of

Equation II-7

$$\log k_2/\alpha = \log (k_a \cdot E) = \log k_a + \log E = \beta pK_a + C$$

enhancement due to the polymeric environment, then the term k_a can be estimated from the reference amines. That is, if the Bronsted equation for the reference amines (Figure II-5) is subtracted from the Bronsted equation for the polymer systems, then E can be determined as a function of pK_a and thus α (Equation II-8). The value of E then can be plotted against pK_a or α (Figure II-9).

The binding aspects of the polymer can be examined more closely via the PNPC reaction. The reactivity of the

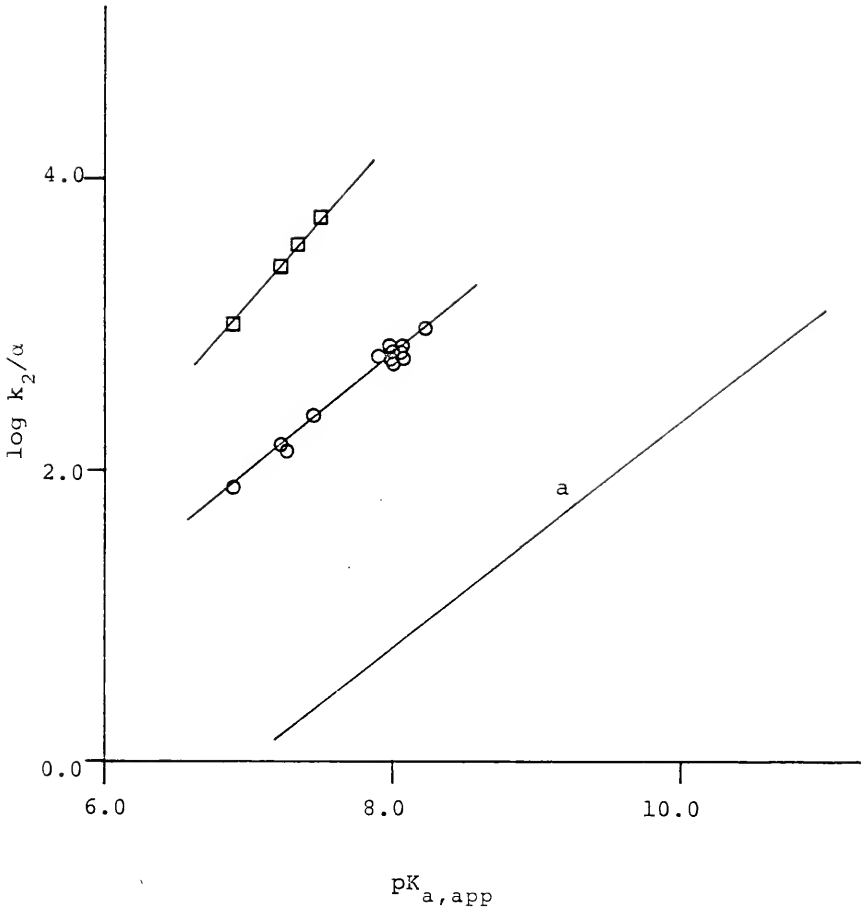


Figure II-8. Bronsted plot for the esterolysis of (□) PNPA PEI-D-NH_2-HCl.

[a] The Bronsted line from Figure II-5 is included as a reference.

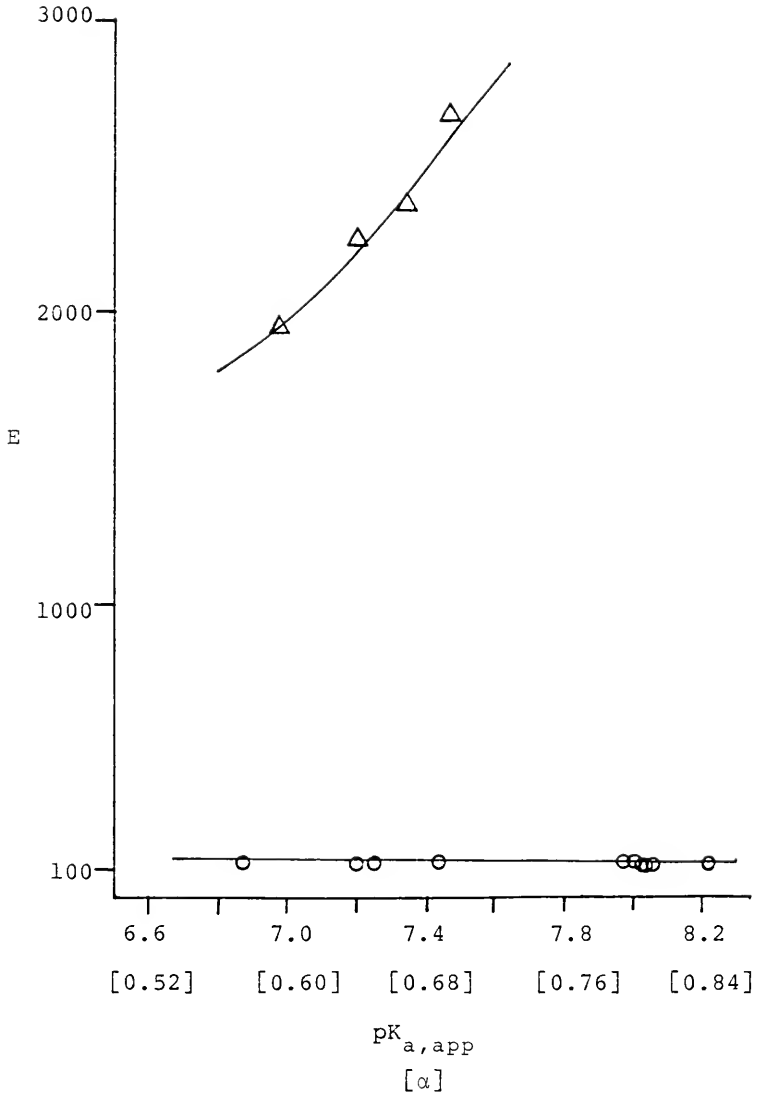


Figure II-9. Plot of E vs. pK_a or [α] for the esterolysis of (Δ)PNPC and (ŏ)PNPA by PEI-D-NH_2-HCl.

Equation II-8

$$\log k_a \cdot E = \beta_p pK_a + C_p \qquad P = \text{polymer system}$$

$$-\log k_R = \beta_R pK_a + C_R \qquad R = \text{reference system}$$

$$\log E = \beta_E pK_a + C_E \qquad E = \text{polymeric enhancement}$$

reference amines with PNPA is used to dissect the rates of the PEI-D-NH_2-HCl reaction with PNPC. In the case of the PNPC aminolysis the dependence of rate on $pK_{a,app}$ is not entirely resolved by the subtraction of the reference equation as in the case of PNPA. The caproate ester and acetate ester aminolyses must proceed by the same mechanism since the only structural change is replacement of a methyl group by a n-pentyl group. Although this change would not be expected to effect the reaction mechanism, the hydrophobic binding properties are greatly affected. Increased hydrophobic binding with reduced charge density then, must be the explanation for the additional pH dependence in the case of PNPC reaction.

The above discussion adequately accounts for the pH dependencies of the reactions of both PNPA and PNPC with PEI-D-NH_2-HCl. However, a significant pH independent enhancement remains unaccounted for. The rate constant for the PEI-D-NH_2-HCl reaction with PNPA at any given pK_a within the range studied is approximately 160 fold larger than a reference amine of the same pK_a. There are several possible explanations for this enhancement, for example, a solvent effect or pH dependent binding. Alternatively, the

apparent enhancement may be due to error in concentration of primary amine and/or in the value of C. Unfortunately there is insufficient data to determine the cause of this enhancement. In spite of insufficient data, this ambiguity does not diminish the importance of the $pK_{a,app}$-rate correlation.

Summary

The reaction of PEI-D-NH_2-HCl with PNPA and PNPC has been shown to proceed via nucleophilic attack of polymeric amine on ester. The rate of reaction is a function of the state of protonation of the polymer which in turn is a function of pH. Correction of the rate constant for fraction of nucleophiles protonated does not lift the pH dependency. However, the pH dependency can be quantitatively dissected by use of the Bronsted relation. The dependence of the PNPA reaction was found to be due to increased nucleophilicity of the polymeric amine with increasing pH. The PNPC reaction exhibits increased binding as well as increased nucleophilicity with increasing pH. The Bronsted relation has not been used directly before to quantitate the electrostatic influence in polyionic nucleophiles. The utility of this approach will be explored further in the next chapter.

CHAPTER III

HYDROLYSIS OF P-NITROPHENYL ESTERS CATALYZED BY POLYMER BOUND HETEROCYCLES

Introduction

In this chapter hydrolysis of p-nitrophenyl esters catalyzed by polymeric systems containing pendent nitrogenous heterocycles will be discussed. As was pointed out in Chapter I, primary amines and nitrogenous heterocycles release p-nitrophenol from p-nitrophenyl esters by the same mechanism. Therefore, the aminolysis reactions of Chapter II are used as models for the discussion. The Bronsted relationship will be employed and its applicability extended.

Polyethylenimine Systems

Preparation

The polymer systems were prepared from PEI-D (described in Chapter II) and the appropriate derivatized heterocycle. PEI-D-Im-HCl was prepared by alkylation of PEI-D with 4(5)-chloromethyl imidazole.[10] PEI-D-Pyr-HCl and PEI-D-APyr-HCl were prepared by reductive amination of PEI-D using, respectively, 4-pyridinecarboxaldehyde and N-(2-pyridyl)-3-aminopropionaldehyde with $NaBH_4$.[11] The fraction of the

polymer derivatized was determined spectrally by NMR and by the C/N ratio from elemental analysis (Table III-1).

Table III-1

% PEI UNITS BOUND TO HETEROCYCLES

Polymer	% CH_2CH_2N Units Bound to Heterocycles	
	Elemental Analysis[a]	NMR[b]
PEI-D-Im-HCl	26±4	26±3
PEI-D-Pyr-HCl	17±4	12±2
PEI-D-APyr-HCl	46±25	25±5

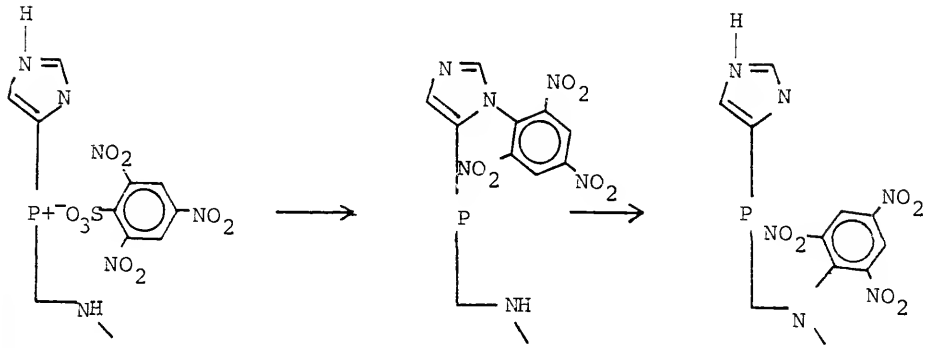
[a]Taken from Table IV-1.
[b]Taken from Table IV-2.

Primary Amine Analysis

As shown in Figure I-5, the reaction rates of PEI-D-NH_2-HCl with PNPA are significantly higher than those of the heterocyclic systems PEI-D-Im-HCl, PEI-D-Pyr-HCl, and PEI-D-APyr-HCl with the same substrate. This observation adds an additional subject for consideration in the interpretation of the kinetic studies for the PEI bound heterocyclic systems. If the PEI bound heterocyclic systems contained residual nucleophilic primary amines, the kinetic data might easily be misinterpreted. That is to say, a small highly reactive fraction of residual primary amines might be the dominant factor in the esterolysis rates measured. Such data might lead one to assign spuriously high rates to

the PEI bound heterocycle esterolysis reaction. It is clear that careful consideration must be given to the primary amine content of PEI bound heterocyclic systems.

Equation III-1



The first choice for primary amine detection in the PEI bound heterocyclic systems might be the trinitrobenzenesulfonate reagent as applied to PEI-D-NH_2-HCl in Chapter II. However the presence of the heterocyclic substituents presents a possible ambiguity in the use of this reagent. Imidazole[28] and pyridines[29] can serve as acyl transfer catalysts in acylation of amines and alcohols. If these heterocycles were to serve a similar function as aryl transfer catalysts[30] (Equation III-1) to secondary amines, the selectivity of the TNBS reagent might well be reduced. Preliminary experiments gave data consistent with this prediction. Based on these observations of TNBS in the PEI-D-Im-HCl systems and the observed secondary amine isopropylation in PEI-D-NH_2HCl (Chapter II), no further attempts were made to find suitable chemical tests. It was decided

that it was unlikely that any reagents would give unambiguous results for the fraction of residual primary amines present in the PEI bound heterocyclic systems.

Having ruled out chemical methods of primary amine detection, kinetic methods were attempted. In Chapter II the exhaustive isopropylation of PEI-D-NH_2-HCl using Acetone/$NaBH_4$ to convert primary amines to secondary amines was discussed. Such a method, although not providing the number of primary amines present, would at least provide a PEI bound heterocyclic system free of primary amines. In Figure III-1 the effects of repetitive isopropylation on the pH rate profile for the PEI-D-Im-HCl esterolysis reaction with PNPA can be seen. PEI-D-Im-HCl was exhaustively isopropylated to PEI-D-Im-Ip-HCl with Acetone/$NaBH_4$. Similar treatment of PEI-D-Im-Ip-HCl produced PEI-D-Im-Ip^2-HCl. The results shown in Figure III-1 illustrate two important features of the PEI-D-Im-HCl. The esterolysis rate is very sensitive to isopropylation. The rate and the pH dependence decrease with increased isopropylation. Further, the rate reduction decreases with isopropylation from 40% reduction for the first isopropylation to 15% reduction for the second isopropylation (at pH=8.00). It follows that a third isopropylation would decrease the rate of reaction by less than 15% (at pH=8.00). A rate reduction of less than 15% is considered insignificant, and the pH rate profile of PEI-D-Im-Ip^2-HCl is assumed to be devoid of a primary amine component.

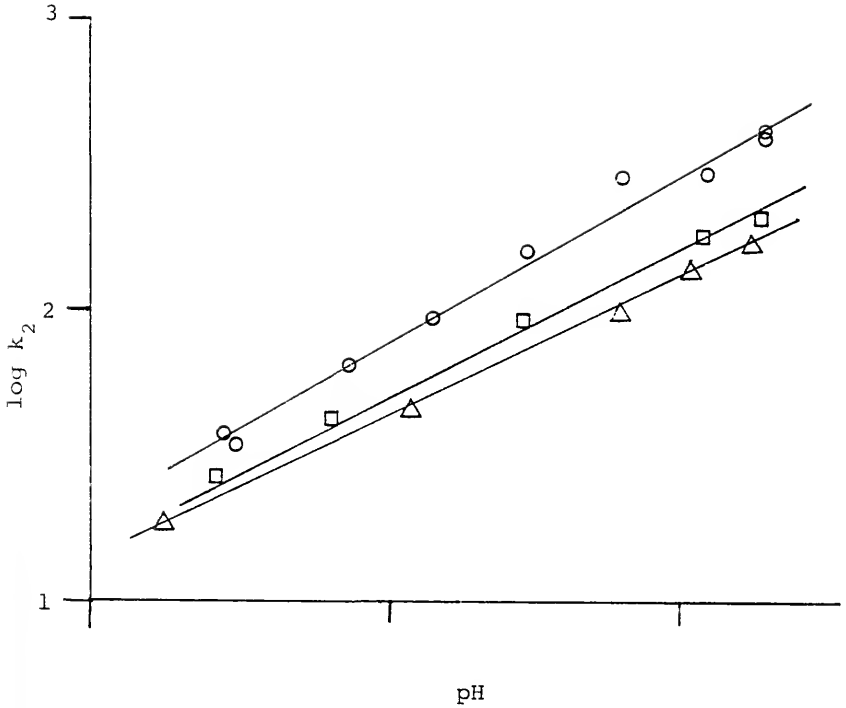


Figure III-1. Effects of repetitive isopropylation of PEI-D-Im-HCl on PNPA esterolysis reactivity. (o) PEI-D-Im-HCl, (□) PEI-D-Im-Ip-HCl, (Δ) PEI-D-Im-Ip^2-HCl.

Further Kinetic Analysis

The PEI-D-Im-Ip^2-HCl will be considered representative of the PEI bound heterocyclic systems. It is clear that the PEI bound pyridine systems show a pH dependency in the pH rate profile just as was shown for the PEI bound imidazole system. Furthermore there is very little difference between the pH rate profiles for the two pyridine containing polymers (Figure I-5). Therefore, the two systems must be reacting by the same mechanism precluding any significant bifunctional effects for PEI-D-APyr-HCl with PNPA.

As in the PEI-D-NH_2-HCl system (Chapter II), the effect of the possible PEI backbone reaction with PNPA must be determined in the reaction of PEI-D-Im-Ip^2-HCl with PNPA. The rate constants k_{2GB}, which are based on concentration of backbone amine for PEI-D-Im-Ip^2-HCl and PEI-D-Ip-HCl, are compared in Figure III-2. The rate constants of PEI-D-Ip-HCl are only 10% of the PEI-D-Im-Ip^2-HCl reaction, at most. Thus the backbone reaction can be ignored for the PEI-D-Im-Ip^2-HCl system. However if the rate of the heterocycle containing polymer was much slower, the backbone reaction would be a serious complication.

The state of ionization of the imidazole in PEI-D-Im-Ip^2-HCl cannot be determined directly by spectrophotometric methods because the amine backbone interferes with the imidazole absorbance. However, the PEI-D-Pyr-HCl system does not suffer such interference, and the pendent pyridine was found to be completely nonprotonated in the pH range studied. Therefore it is reasonable to assume that the

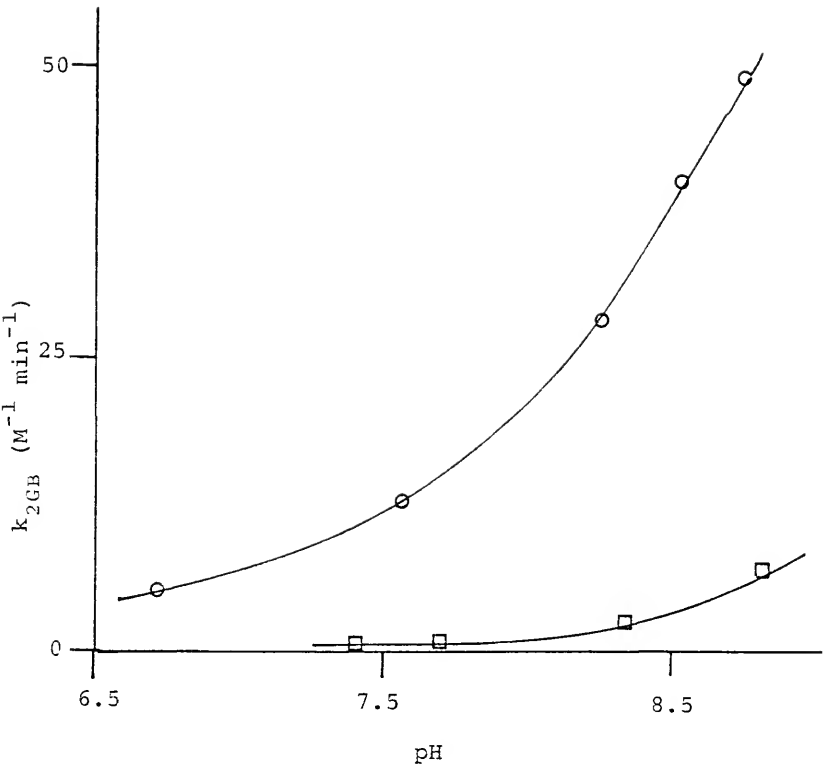


Figure III-2. Comparison of PNPA esterolysis rates for (o) PEI-D-HCl and (□) PEI-D-Ip-HCl.

imidazole in PEI-D-Im-HCl is unionized throughout the large part of the range.

Since the imidazole is virtually nonprotonated in the region of interest, its pK_a is undefined in this region (Chapter I). Without pK_a data a Bronsted plot cannot be constructed. Even though the pK_a data are not available it is valid to assume that basicity increases with pH just as it does in the PEI-D-NH_2-HCl system (Chapter II). It was found in Chapter II that the nucleophilicity, and hence the reactivity, of the PEI-D-NH_2-HCl system was dependent upon the pK_a or basicity of the polymer. By analogy one would then expect that the reactivity of the PEI-D-Im-Ip^2-HCl would increase with pH as is observed in Figure III-1.

Summary

There are several important statements to be made concerning the above observations and conclusions. The fact that the rate of reaction is so dependent on isopropylation indicates that there may well be a significant contribution to the esterolysis rate by residual primary amines (backbone reaction) in the PEI-D-Im-HCl polymer. The residual primary amine content in the presence of imidazole is difficult to determine as discussed earlier. Klotz et al.[12] prepared a system very similar to PEI-D-Im-HCl. Their polymer only differed in imidazole content (15%) and degree of ionization. They tested for primary amine content by the ninhydrin method and found that it did not exhibit the characteristic ninhydrin color. This result led to the conclusion that

there were no primary amines present. Such a result is not surprising, as the parent system (PEI-D-NH_2-HCl) which contains 20% primary amine also does not exhibit the expected "Ruheman's Purple" (a deep purple blue) of ninhydrin with primary amines.[29] The parent system does produce a dull brown color, possibly an oxidation product, but it in no way resembles the true ninhydrin color. Further, it would be expected that their polymer would contain even more primary amines than PEI-D-Im-HCl due to a smaller fraction of the backbone alkylated with methyleneimidazole units, 15% as opposed to 29%. The presence of primary amines in the polymer studied by Klotz et al.[12] might indicate a significant primary amine component in the presumed imidazole acylation rate.

Another important point is the ability to qualitatively predict a pH dependence for the PEI-D-Im-Ip^2-HCl system based on the Bronsted correlation found for PEI-D-NH_2-HCl. This example begins to show the usefulness of this application of the Bronsted relationship. The application to non PEI systems follows.

Non Polyethylenimine Homopolymer Systems

Unlike the PEI bound imidazole above, there are some examples of polymer bound heterocycles for which Bronsted plots can be constructed. Letsinger and Saveride[25] studied poly-4-vinylpyridine catalyzed 2,4-dinitrophenyl acetate (DNPA) hydrolysis. They found that although 4-methylpyridine produced the expected linear, rate vs α plot, the

poly-4-vinylpyridine exhibited a curved plot (Figure III-3). They qualitatively attributed the curvature in the polymer reaction to decreased nucleophilicity with increasing protonation (decreasing α). Overberger et al.[6] found very similar results in poly-4(5)-vinylimidazole versus imidazole catalysis of hydrolysis of PNPA (Figure I-1a). They attributed the curvature to several possible bifunctional mechanisms as discussed in Chapter I. The rate vs α plot for the reaction of PEI-D-NH_2-HCl with PNPA is shown in Figure III-4. Similar to the plots for poly-4(5)-vinylimidazole (Figure I-1a) and poly-4-vinylpyridine (Figure III-3) the rate vs α plot for PEI-D-NH_2-HCl is curved. Since the Bronsted relationship was able to quantitatively account for the PEI-D-NH_2-HCl pH dependence of PNPA esterolysis, it is reasonable to apply the relationship to the above reactions of poly-4-vinylpyridine (Figure III-5) and poly-4(5)-vinylimidazole (Figure III-6). As in the case of the PEI-D-NH_2-HCl reaction with PNPA (Figure II-8) the Bronsted plots are linear. The plot for the poly-4-vinylpyridine system produces a large value of β (1.3); however, small molecule pyridine β values are typically larger than 1.[21] Furthermore, the slopes of the pH-rate profiles for the pyridine containing PEI- systems, 0.69 and 0.74 (Figure I-3 and Figure I-2), were larger than the slope of the imidazole system (0.56, Figure I-4). The poly-4(5) vinylimidazole Bronsted slope, 0.8, is precisely that expected from the portion of the small molecule imidazole plot shown on the same plot. Therefore the interpretation of Letsinger and

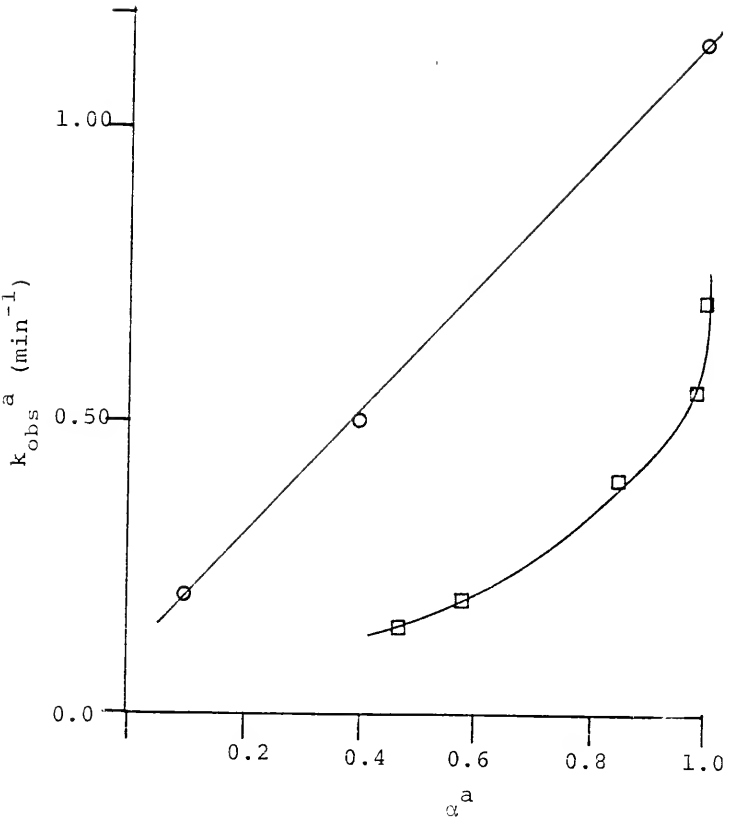


Figure III-3. Plot of k_{obs} against α for the esterolysis of 2-4-dinitrophenyl acetate by (□)poly-4-vinyl-pyridine and (o)pyridine.

[a] Values of k_{obs} and α determined that 36.8°C in 50% aqueous ethanol.[25]

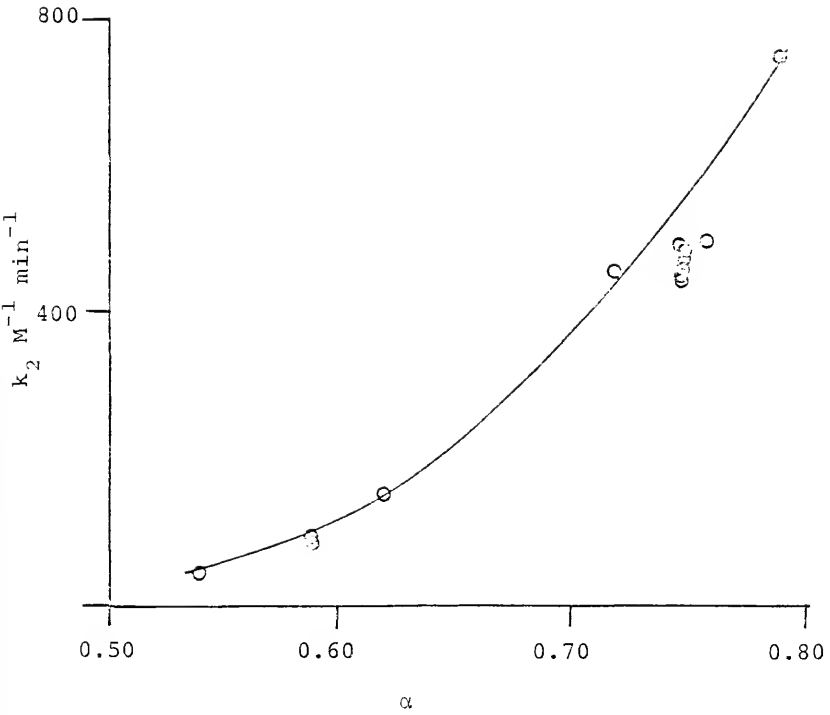


Figure III-4. Plot of k_2 against α for the esterolysis of PNPA by PEI-D-NH_2HCl.

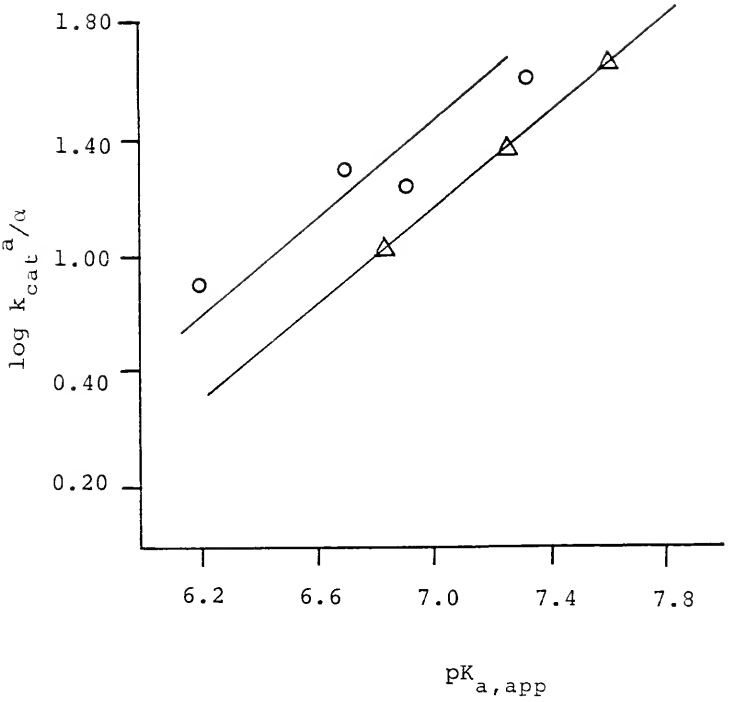


Figure III-5. Bronsted plot for the esterolysis of PNPA by (Δ)poly-4(5)-vinylimidazole and (o)imidazoles.

[a] Values of k_{cat} and $pK_{a,app}$ determined at 30°C in 28.5% ethanol.[7,27]

[b] From Figure II-6.

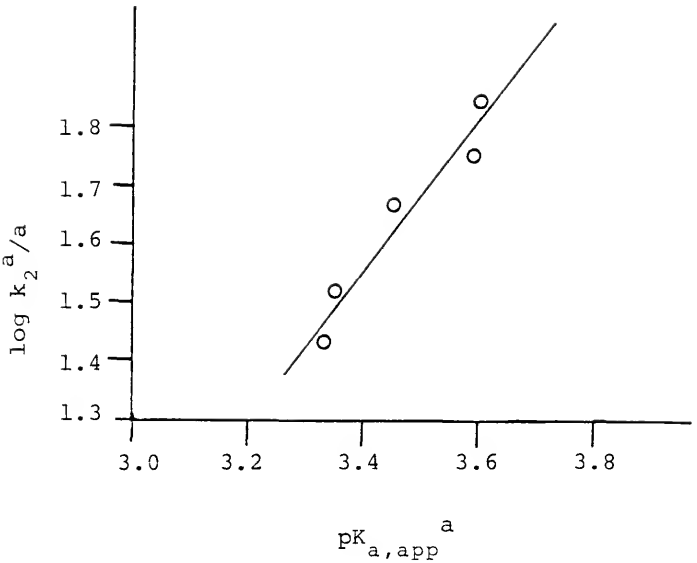


Figure III-6. Bronsted plot for the esterolysis of 2,4-dinitrophenylacetate by poly-(4)-vinylpyridine.

[a] Values of pK_a and k_2 determined at 36.8°C in 50% aqueous ethanol.[25]

Saveride[25] would seem to be sufficient for the poly-4(5)-vinylimidazole system. That is, the curvature in the rate vs α plot is due to increasing pK_a of the nucleophile. The increase in pK_a is in turn due to the reduction of the positively charged electrostatic field as α increases. At low values of α the highly charged polymer increases the energy required for formation of additional positive charge. As α increases the charge on the polymer decreases, thereby reducing the energy required for formation of positive charge and increasing pK_a and reaction rate.

Conclusion

The correlation of $pK_{a,app}$ with reaction rate is quite effective in quantifying pH dependencies in polymer esterolysis reactions for pH regions in which the nucleophile is partially ionized. If the nucleophile is completely nonprotonated the $pK_{a,app}$ cannot be determined. However, the $pK_{a,app}$ and thus nucleophilicity are dependent on the state of ionization of the polymer. Therefore it is reasonable to predict that, if polymeric functions other than the nucleophile undergo ionization with pH changes, the nucleophilicity (and thus rate) will be pH dependent. The pH dependencies of the heterocycle containing PEI systems can be explained as a pK_a dependence. Bifunctional effects were ruled out for the pyridine containing PEI systems due to the similarity of the pH-rate profiles. It also seems very unlikely that the imidazole containing PEI reaction with PNPA has a significant bifunctional component in view

of the correlation of the $pK_{a,app}$ and rate for poly-4(5)-vinylimidazole. As pointed out in Chapter I this conclusion does not necessarily rule out the possibility of a bifunctional mechanism for the polymer systems discussed. The fault may be in the choice of the ester.

Our application of the Bronsted plot to quantitate pH dependencies is a very important development in the field of polymer esterolysis. It has been shown that polymers of varying make up have different reactivities. For example, Shimidzu et al.[31] have plotted esterolysis rate constants against the intrinsic pK_a (see Appendix) for a series of imidazole containing polymers. The work of Shimidzu shows clearly that increasing acrylic acid content in a series of acrylic acid and vinylimidazole copolymers increases the imidazole pK_a and thus the esterolysis rate. However, up to now Bronsted correlations for polymer systems have been for series of polymers. Such correlations cannot account for pH dependencies as can Bronsted correlations for a single polymer system plotting $pK_{a,app}$ vs rate constants. This type of Bronsted correlation has proven valuable in explaining both the pH dependencies generated by the research herein as well as that of others. Perhaps the best test of a tool is how well it works. The correlation of $pK_{a,app}$ to esterolysis rate constants has been shown here to work well for the polymer systems tested.

CHAPTER IV

EXPERIMENTAL

Introduction

Melting points and boiling points are uncorrected unless otherwise noted. Melting points were determined with a Thomas Hoover Unimelt capillary melting point apparatus. Boiling points were determined by conventional techniques. Nuclear Magnetic Resonance spectra were recorded using a Varian A60-A spectrometer. Chemical shift (δ) data were reported in parts per million (ppm) from the appropriate internal reference, either tetramethylsilane (TMS), 2,2-dimethyl-2-silapentane 5-sulfonic acid (DDS), or water d_1 (HOD). Solvent evaporation was performed at reduced pressure using a Buchler Instruments flash evaporator. Lyophilisation or freeze drying was carried out using an apparatus and technique similar to that described by Vogel.[32] The aqueous dialyses were carried out in a 3 liter filter flask connected to a deionized water tap and equipped with a stir bar. The polymer solutions to be dialyzed were transferred to dialysis bags prepared from Union Carbide (36 100 ft dialysis membrane) tubing according to the method of Gabbay et al.[33] and generously supplied by Shau-Fong Yen. In the case of

Dialysis against a stream of deionized water, the tap was opened to allow a moderate flow of water through the flask while stirring. In the case of dialysis against 0.1 M HCl, the tap was closed, and 30 ml concentrated hydrochloric acid was added. When dialyzing against ethanolic solvents the bag was stirred in a 500 ml flask with the solvent. Temperature control was provided by a Lauda K-2/R thermostat. All UV-visible spectrophotometric measurements were made on a Cary 17D spectrophotometer. The pH measurements were made with a Beckman Research pH meter in conjunction with a Radiometer GK 2321C electrode.

Syntheses

Dodecyl Polyethylenimine PEI-D

Dow PEI 600 polyethylenimine solution (128 g) was freeze dried for 8 hr and then allowed to warm to room temperature while maintaining vacuum (0.05 torr) for an additional 36 hr. The gelatinous material (49 g, 1.2 mol) was dissolved in 470 ml argon saturated anhydrous ethanol. Dodecyl iodide (35 g, 0.12 mol) in 5.0 ml of anhydrous ethanol was added to the polymer solution. The reaction flask was sealed and maintained at 45°C for 120 hr. The reaction mixture was quantitatively transferred to a one liter volumetric flask and diluted to the mark with argon saturated anhydrous ethanol. This solution was stored under argon and used as a stock solution in the following preparations (1.2 M in ethylenimine units).

Dodecyl Polyethylenimine Hydrochloride PEI-D-NH_2-HCl

A solution of dodecyl polyethylenimine was prepared by diluting PEI-D (25 ml, 0.030 mol) up to 150 ml with absolute ethanol. This solution was added carefully to a 500 ml beaker containing 5 ml concentrated hydrochloric acid in 50 ml absolute ethanol at 0°C. The resultant salt was filtered and washed with absolute ethanol.

Dodecyl-4-methylenepyridine-polyethylenimine Hydrochloride PEI-D-Pyr-HCl

Freshly distilled 4-pyridinecarboxaldehyde (BP 191-192°C under N_2, 0.96 g, 0.0090 mol) was dissolved in 25 ml absolute ethanol in a 125 ml erlenmeyer flask equipped with a stir bar. PEI-D (25 ml, 0.030 mol) was added to the flask. The cloudy suspension was stirred for 2 hr under N_2. A 50 ml solution of $NaBH_4$ (0.13 g, 0.0022 mol) was added to the flask at 0°C. The reaction was stirred for 2 hr. The reaction mixture was transferred to 4 tubes. One milliliter concentrated hydrochloric acid was added to each tube to form the polymer salt, and the tubes were then centrifuged. The precipitated polymer salt was washed twice with absolute ethanol. The polymer salt was dialyzed against 500 ml absolute ethanol twice. The ethanolic dialysate was passed through a sephadex LH-20 column. The eluant was transferred into 4 tubes. The salt was reprecipitated by addition of one milliliter concentrated hydrochloric acid to each tube. The tubes were centrifuged, and the salt was washed twice with absolute ethanol. The polymer salt was dryed producing a white powder (1.12 g, 0.00966 mol, 32%).

Dodecyl-N-(2-pyridyl)-3-propylamine-polyethylenimine Hydrochloride PEI-D-APyr-HCl

Ten milliliters of an absolute ethanol solution of N-(2-pyridyl)-3-aminopropionaldehyde hydrochloride (0.65 g, 0.0031 mol) was prepared in a 125 ml erlenmeyer flask with gentle heating. A stir bar and PEI-D (10 ml, 0.012 mol) was added to the flask which was stirred for one hour under N_2. A 10 ml absolute ethanol solution of $NaBH_4$ (0.092 g, 0.0024 mol) was added to the reaction mixture. After stirring for an hour, the reaction mixture was transferred to a tube. The polymer salt was precipitated by addition of one milliliter concentrated hydrochloric acid. After centrifuging, the polymer salt was washed twice with absolute ethanol. The polymer salt was dialyzed against a stream of deionized water for 24 hr. The polymer salt was then dialyzed against 500 ml absolute ethanol overnight. The dialysate was passed through a sephadex LH-20 column. The polymer salt was reprecipitated by the addition of one milliliter of concentrated hydrochloric acid. The polymer salt was dried producing an off-white solid (0.35 g, 0.0023 mole, 19%).

Dodecyl-4(5)-methylenimidazole-polyethylenimine Hydrochloride PEI-D-Im-HCl

To a solution of PEI-D (25 ml, 0.030 mol) in a sealable tube was added 4(5)-chloromethylimidazole hydrochloride (1.59 g, 0.10 mol), triethylamine (2.5 g, 0.025 mol) and a stir bar. The tube was sealed and maintained at 65°C in an oil bath for 36 hr. After cooling the tube was opened and the contents transferred to two test tubes. One milliliter of concentrated hydrochloric acid was added to each tube forming a heavy

precipitate. The tubes were centrifuged and the supernatant decanted. The precipitates were washed with absolute ethanol, centrifuged, and the supernatant decanted. The precipitate was dissolved in 20 ml deionized water and dialyzed against a stream of deionized water for 72 hr. The polymer was then dialyzed with 500 ml absolute ethanol three times. The polymer solution was removed from the dialysis bag and passed through a sephadex LH-20 column. The salt was reprecipitated with 4 ml concentrated hydrochloric acid. The salt was washed twice with absolute ethanol. The polymer salt was dried producing an off-white solid (1.75 g, 0.0143 mol, 48%).

Dodecyl-4(5)-methylenimidazole-isopropyl-polyethylenimine Hydrochloride PEI-D-Im-Ip-HCl

PEI-D-Im-HCl (0.0300 g) was dissolved in 30 ml of water. This solution was treated with solid $NaBH_4$ to bring the pH up to approximately 7. After freeze drying the polymer was suspended in 50 ml absolute ethanol containing one gram acetone by the dropwise addition of 6 N hydrochloric acid. After one hour $NaBH_4$ (0.4 g, 0.01 mol) in 10 ml absolute ethanol was added causing precipitation. A routine of suspension of the polymer with 6 N hydrochloric acid followed by addition of acetone (5 g, 0.8 mol), then one hour later addition of $NaBH_4$ (0.4 g, 0.01 mol) in 10 ml absolute ethanol was followed twice. The polymer was reacidified with 6 N hydrochloric acid and allowed to stir for 12 hr to complete hydrolysis of the residual $NaBH_4$. The solution was dialyzed for 24 hr. The polymer solution was centrifuged then filtered. The filtrate was freeze dried producing a white solid (0.281 g, 94%).

Dodecyl-4(5)-methylenimidazole-isopropyl-isopropyl-polyethylenimine Hydrochloride PEI-D-Im-Ip^2-HCl

PEI-D-Im-Ip-HCl (0.098 g) was dissolved in 10 ml of water. This solution was heated with solid $NaBH_4$ to bring the pH up to approximately 7. After freeze drying the polymer was stirred in 20 ml absolute ethanol containing one gram acetone for one hour. The polymer was suspended by dropwise addition of 6 N hydrochloric acid and 5 ml absolute ethanol containing one gram acetone was added. After one hour $NaBH_4$ (0.4 g, 0.01 mol) was added in 10 ml of absolute ethanol followed by suspension of the polymer salt with 6 N hydrochloric acid one hour later. The reaction mixture was stirred under a stream of N_2 overnight. The polymer was dialyzed against a stream of deionized water for 24 hr followed by 0.1 N hydrochloric acid for 3 hr. The polymer solution was centrifuged, filtered then freeze dried producing a white solid (0.062 g, 63%).

4(5)-Chloromethylimidazole Hydrochloride

4(5)-Chloromethylimidazole hydrochloride was prepared according to the procedure of Turner et al.[34] from 4(5)-hydroxymethylimidazole and thionyl chloride (mp 138-140°C, lit mp 138-141°C).

4(5)-Hydroxymethylimidazole Hydrochloride

In a mixture of 50 ml concentrated hydrochloric acid, 125 ml water and 250 ml benzene 4(5)-hydroxymethylimidazole picrate (69 g, 0.21 mol available from Eastman) was dissolved with heating. The benzene which developed a

yellow color was decanted. The aqueous solution was continuously extracted with 125 ml benzene layer for 9 hr. After decanting the benzene layer, the aqueous layer was evaporated under vacuum at 70°C. The yellow brown residue was recrystalyzed from ethanol ether (23 g, 81% mp 103-106°C, lit mp 107-109°C).

3-Bromopropanal Dimethyl Acetal

3-Bromopropanal dimethyl acetal was prepared according to the procedure of Pineau:[35] bp 52°C/10 mm Hg, lit[35] bp 59°C/12 mm Hg; nmr $CDCl_3$ δ 2.12 (doublet of triplets, J=5 Hz, J=8 Hz, 2H), 3.36 (singlet, 6H), 3.43 (triplet, J=8 Hz, 2H), 4.53 (triplet, J=5 Hz, 1H).

N-(2-pyridyl)-3-aminopropanal Dimethyl Acetal

The synthesis of N-(2-pyridyl)-3-aminopropanal dimethyl acetal was carried out according to the procedure described by Reynaud et al.[36] The product was an amber oil: bp 154°C/10 mm Hg, lit bp 153°C/12 mm Hg; nmr ($CDCl_3$) δ 1.92 (quartet, 2H), 3.32 (singlet, 6H), 3.35 (quartet, 2H), 4.50 (triplet, 1H) 4.75-5.20 (mult., 1H) 6.25-6.75 (mult, 2H), 7.20-7.55 (mult., 1H), 8.05 (doublet of doublets, 1H).

N-(2-pyridyl)-3-aminopropanal Hydrochloride

N-(2-pyridyl)-3-aminopropanal dimethyl acetal (1.3 g, 0.0064 mol) was dissolved in 5 ml concentrated hydrochloric acid and heated on the steam cone for one hour. The solvent was evaporated and the brown residue was dried under vacuum (0.40 g, 0.0018 mol, 28%); mp 172-172.5°C.

p-Nitrophenyl Acetate PNPA

P-nitrophenyl acetate was prepared according to the procedure of Bender and Nakamura.[37] The solid p-nitrophenyl acetate was recrystalyzed twice from ethanol; mp 78-78.5°C, lit mp[37] 77.5-78°C.

p-Nitrophenol Caproate PNPC

P-nitrophenol (14 g, 0.1 mol) was dissolved in 25 ml dry pyridine. The pyridine solution was added to caproylchloride (13 g, 0.1 mol) in a 100 ml round bottom flask equipped with a reflux-condenser and stir bar. The reaction was refluxed for 15 hr. After cooling, the contents of the reaction flask were added to 40 ml of ice water. The resulting oily layer was separated and the aqueous layer extracted twice with 25 ml diethyl ether. The combined oil and ethereal extract was washed 3 times with 50 ml water, 5 times with 50 ml 5% aqueous hydrochloric acid, and 6 times with 5% aqueous sodium bicarbonate (each washing had to be salted out). The ethereal layer was dried and stripped producing 17 g of crude material. The crude product was distilled producing a viscous oil (13 g, 0.055 mol, 55%); bp 150°C/2 mm Hg, lit[38] bp 145°C/1 mm Hg.

Composition of PEI Derivatives by Elemental Analysis

Elemental analysis data were used to calculate the values of D, (fraction CH_2CH_2N units dodecylated), z (fraction of CH_2CH_2N units bearing a heterocycle), mole unit weight (average weight of a mole of CH_2CH_2N units after

derivitization), and α initial (in the case of PEI-D-NH_2HCl). The C/N ratio was used to calculate both D and Z. The carbon to nitrogen weight ratio is equal to the C/N ratio from elemental analysis. Therefore the number of carbons per unit (2 from the CH_2CH_2N unit, 12D from the dodecyl group, and ZX from the heterocycle, X is the number of carbons in the heterocycle) divided by the number of nitrogens per unit (one from the CH_2CH_2N unit and ZY from the heterocycle, Y is the number of nitrogens in the heterocycle) multiplied by the C/N atomic weight ratio is equal to the C/N ratio from elemental analysis (Equation IV-1). This equation is readily rearranged to solve for either D or Z. In the case of PEI-D-NH_2-HCl the heterocycle terms drop out and the equation is in a single unknown, D. For the PEI derivatives containing heterocycles the value of D calculated for PEI-D-NH_2-HCl was used. The error limits in these calculations are based on an assumed error of 0.2 in the elemental analysis. Values of C/N were calculated using C+0.2/N-0.2 and C-0.2/N+0.2 which were substituted in the appropriate equations to calculate error limits. The mole unit weight was calculated from the relationship that % of an element in a compound is equal to the weight of that element in the compound divided by the formula (units) weight of the compounds multiplied by 100 (Equation IV-2). The elemental analysis values for carbon and nitrogen together with the calculated values for D and Z were used for these calculations (Equation IV-30). The error calculation for the unit

mole weight was based on the difference between the average from the carbon and nitrogen calculations and the highest possible value from the carbon based calculation and lowest possible value for the nitrogen based calculation consistent with the assumed error of ±0.2 in elemental analysis data. The value of α initial was calculated for the PEI-D-NH_2-HCl titration based on the assumption that all analyzed chlorine was present as the hydrochloride salt. The ratio of gram atoms of chlorine to gram atoms of nitrogen in the polymer represents the fraction of CH_2CH_2N units protonated, 1-α initial (Equation IV-4). The error calculation for α initial was made assuming an error of 0.02 in elemental analysis data as in previous calculations (Table IV-1).

Equation IV-1

$$C/N = \frac{2 + 12D + ZX}{1 + ZY} \left[\frac{12.01}{14.01}\right]$$

$$D = \left[C/N \frac{14.01}{12.01} (1 + ZY) - 2 + 2X\right] \frac{1}{12}$$

$$D = \left[C/N \frac{14.01}{12.01} - 2\right] \frac{1}{12}$$

$$Z = \frac{C/N \frac{14.01}{12.01} - 2 - 120}{X - C/N \frac{14.01}{12.01} Y}$$

Equation IV-2

$$\% \text{ element} = \frac{\text{weight of element X 100}}{\text{formula weight (mole unit weight}}$$

Table IV-1

COMPOSITION OF PEI DERIVATIVES FROM ELEMENTAL ANALYSIS DATA

Elemental Analysis Data[a]	Polymer Systems				
	PEI-D-NH_2-HCl	PEI-D-Ip-HCl	PEI-D-Im-HCl	PEI-D-APyr	PEI-D-Pyr-HCl
C	42.17±0.02	46.27±0.05	44.99±0.03	45.66±0.06	45.91±0.05
H	7.94±0.01	9.64±0.01	7.85±0.03	8.32±0.02	8.50±0.01
N	14.66±0.02	12.46±0.03	18.15±0.03	14.52±0.02	14.29±0.02
Cl	32.64	27.18±0.04	25.29±0.04	24.57±0.07	27.12±0.04
Total	96.41±0.05	95.53±0.05	96.27±0.12	93.07±0.15	95.81±0.01
Calculated Parameters[d]					
C/N	0.88±0.05	3.71±0.08	2.48±0.04	3.14±0.05	3.21±0.06
D[b]	0.113±0.005	-	-	-	-
Z[c]	-	0.32±0.04	0.26±0.04	0.46±0.25	0.17±0.04
Mole Unit Wt.	96±1	115±1	116±5	190±50	115±5
α initial	0.15±0.02	-	-	-	-

a All elemental analysis in duplicate except Cl for PEI-D-NH_2HCl.
b D, fraction of CH_2CH_2N dodecylated.
c Z, fraction of CH_2CH_2N bearing heterocycle.
d Parameters calculated from Equations IV-1, IV-2, IV-3 and IV-4.

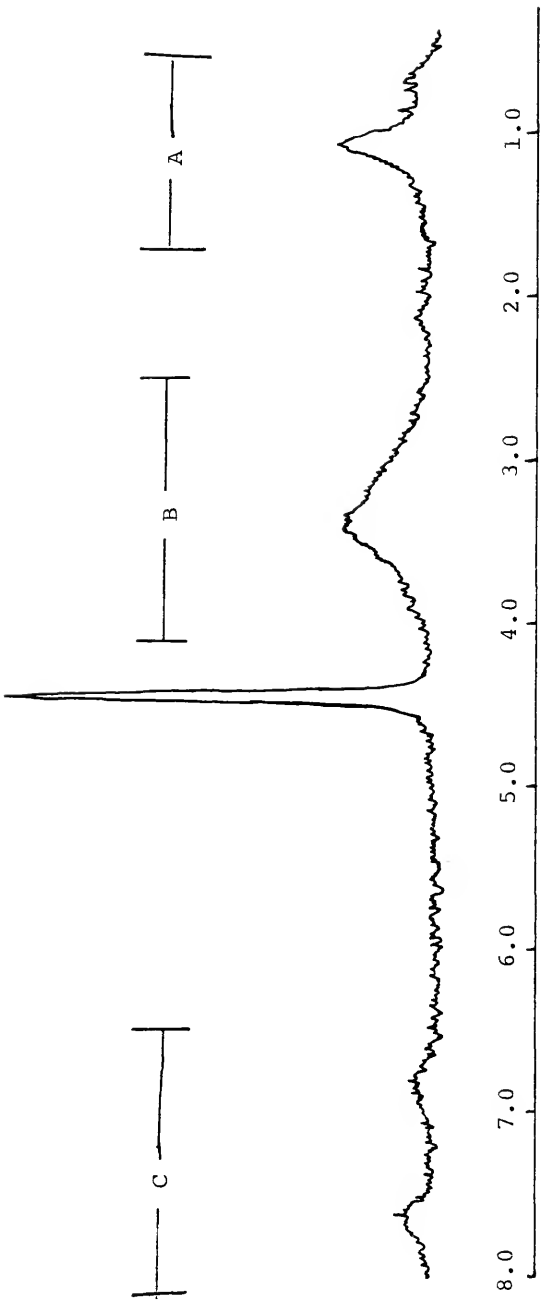


Figure IV-1. Typical NMR spectrum of a PEI-D derivative (PEI-D-APyr-HCl). The dodecyl group resonance is in region A, the CH_2CH_2N unit in region B and the heterocyclic group in region C.

TABLE IV-2

FRACTION OF PEI UNITS ALKYLATED FROM NMR SPECTRAL DATA

	PEI-D-NH_2-HCl	PEI-D-Im-HCl	PEI-D-APyr-HCl	PEI-D-Pyr-HCl
Region[a] A	1.05-1.47	0.60-1.47	0.33-1.42	0.47-1.47
Area[b] A/H	1.61-0.04	6.7-0.3	2.3-0.1	0.60-0.05
Region B	1.93-3.88	2.47-3.63	2.50-4.00	2.30-3.72
Area B/H	15.8-0.5	-	-	-
Region C		7.00-8.83	6.33-8.08	7.30-8.83
Area C/H	-	17.8-1.3	5.7-0.8	0.81-0.23
Fraction[c] Alkylated	0.10-0.01	0.26-0.03	0.25-0.05	0.12-0.02

a See Figure IV-1, Chemical shift δ based on
b Integrated area divided by the number of hydrogens in that specific group.
c Column 1 refers to dodecyl group alkylation. Columns 2, 3 and 4 refer to alkylation by heterocycles.

Equation IV-3

$$\text{mole unit weight} = \frac{(2 + 12D + ZX)\ 12.01}{\%C \times 0.01}$$

$$\text{mole unit weight} = \frac{(1 + ZY)\ 14.01}{\%N \times 0.01}$$

Equation IV-4

$$1 - \alpha = \frac{\%Cl/35.45}{\%N/14.01}$$

Composition of PEI Derivatives by NMR Spectral Analysis

The NMR spectra and peak areas (see Figure IV-1) of the polymer salts were recorded (Table IV-2). The integrated areas per hydrogen (for example, area of imidazole peak divided by 2) was used to calculate the extent of substitution on the polymer. In the case of PEI-D-NH_2-HCl the fraction of CH_2CH_2N units alkylated was determined by the ratio of dodecyl area per hydrogen to the CH_2CH_2N area per hydrogen (corrected for the N terminal methylene of the dodecyl group). In the heterocycle containing systems the dodecyl peak was used as a reference instead of the CH_2CH_2N peak.

Determination of Primary Content of PEI Derivatives Using Trinitrobenzene Sulfonate (TNBS)

The primary amine content of PEI derivatives was determined using trinitrobenzenesulfonate (TNBS) by a method similar to that used by Johnson and Klotz.[10] To a 25 ml

volumetric flask was added 0.100 ml of an aqueous PEI solution (0.0001 units mol), 5 ml 4% aqueous sodium bicarbonate solution, and one milliliter 0.1% aqueous TNBS solution. The flask was heated 0.5 hr at 37°C, cooled to room temperature, filled to the mark with galatial acetic acid, and the absorbance measured at 340 nm. The percentage of residual primary amine was calculated based on the extinction coefficient used by Johnson and Klotz,[10] 12000 M^{-1} (Table IV-3).

TABLE IV-3

PRIMARY AMINE CONTENT OF PEI DERIVATIVES USING TNBS

PEI Derivative	Absorbance	Unit Mole Concentration of PEI	% Primary Amine
PEI-DNH_2-HCl	0.972±0.013	4.08×10^{-4}	19.8±0.4
PEI-D-Ip-HCl	0.058±0.001	3.44×10^{-4}	1.4±0.2
Blank	0.007±0.007	-	

Primary Amine Detection by Ninhydrin

The efficacy of primary amine detection by ninhydrin was compared for PEI-D, ethylenediamine, and glycine according to the procedure suggested by Pasto and Johnson.[39] A strip of filter paper and a strip of chromatogram sheet (silica gel) was spotted with 0.1 M aqueous solutions of the amine systems to be compared. After oven drying the strips were

sprayed with 0.25% ninhydrin solution and developed for five minutes at 100°C. Additionally 0.1 M solutions of the amines to be compared were treated with one milliliter of 0.25% ninhydrin solution and gently warmed for 0.5 hr. The glycine results were those expected: blue solution[39] and purple blue spots.[40] Ethylenediamine did not react with the ninhydrin and produced no color changes; similar results have been previously reported.[41] The PEI-D spots were brown and the solution was yellow.

Relative Concentrations of Imidazole Containing Polymer Solutions

The relative concentrations of stock solutions of the three imidazole containing polymers PEI-D-Im-HCl, PEI-D-Im-HCl, and PEI-D-Im-Ip^2-HCl used for kinetics were determined spectrophotometrically. A series of 7 solutions were prepared from the PEI-D-Im-HCl stock solution in 1.0 M hydrochloric acid. The absorbencies of these solutions ranging in concentration from 2.24×10^{-5} to 28.0×10^{-5} molar in imidazole units were measured at 210 nm and 25.0±0.3°C. A Beer's law plot was constructed from this data. The least squares line passed through 0 (within experimental error r=0.99998) and had a slope, extinction coefficient, of 6.79×10^3 M^{-1}. Solutions (2 each) of the isopropylated imidazole containing polymers were prepared from the respective stock solutions in the same way (ca 10^{-5} M) and their absorbancies measured. The concentrations of the stock solutions were calculated from the extinction coefficient of the PEI-D-Im-HCl system. The

concentrations of PEI-D-Im-Ip-HCl and PEI-D-Im-Ip^2-HCl solutions then were determined relative to the PEI-D-Im-HCl.

Potentiometric Titration of PEI-D-NH_2-HCl

The PEI-D-NH_2-HCl (0.0931 g) was dissolved in 50.0 ml of 0.100 M KCl after drying under vacuum for 10 days. The solution (0.0192 M in CH_2CH_2N units) was titrated with CO_2 free KOH (0.09977 N) at 25.0±0.3°C under a nitrogen atmosphere. After each addition of titrant the pH was recorded upon equilibration.

The values of $pK_{a,app}$ and α were calculated from the titration data. The value of α at each pH was determined by a modification of the method suggested by Albert and Serjeant[42] to calculate the ratio of nonprotonated species (Equation IV-5). The value of KOH refers to the initial

Equation IV-5

$$\alpha = \frac{[KOH] + \alpha_{int}\,[CH_2CH_2N] - 10^{-pH} + 10^{pH-14.00}}{[CH_2CH_2N] + 10^{-pH} - 10^{pH-14.00}}$$

concentration of potassium hydroxide. That is, the total moles of titrant added to bring the pH to a given value divided by the volume of the solution. The value of α_{int} is the fraction of nonprotonated CH_2CH_2N units in PEI-D-NH_2-HCl as prepared and determined from the Cl/N ratio. The exponential terms in pH serve to correct the hydroxide ion concentration for water ionization. The value of $pK_{a,app}$ was calculated at each value of pH by use of the Henderson-Hasslebalch equation[19] (Equation II-3, Table IV-4).

TABLE IV-4

POTENTIOMETRIC TITRATION DATA FOR PEI-D-NH_2-HCl

Titrant (ml)[a]	pH	α	$pK_{a,app}$
0.000	3.00	0.199	3.61
0.500	3.19	0.233	3.71
1.000	3.47	0.269	3.91
1.640	4.14	0.321	4.46
2.000	4.64	0.356	4.89
2.500	5.45	0.407	5.62
3.000	6.05	0.459	6.12
3.500	6.62	0.510	6.60
4.000	7.17	0.562	7.06
4.500	7.63	0.614	7.43
5.000	8.00	0.666	7.70
5.500	8.32	0.718	7.91
6.000	8.64	0.770	8.12
6.500	8.96	0.821	8.30
7.000	9.34	0.873	8.50
7.500	9.75	0.922	8.67
8.000	10.25	0.967	8.78
8.500	10.73	0.996	8.33

a 0.09977±0.09% CO_2 free aqueous KOH.

Equation II-3

$$pK_a = pH + \log \frac{1-\alpha}{\alpha}$$

Preparation and Standardization of CO_2 Free 0.1 N KOH Titrant

An ampule of J. T. Baker Dilut-it was quantitatively transferred to a 1000 ml volumetric flask as per enclosed instructions. The contents of the volumetric flask were diluted to the mark with "boiled out" water. The titrant was standardized with oven dried (120°C, one hour) primary standard pottasium hydrogen phthalate (KHP). The standardizatiocn was repeated seven times (N=0.09977±0.09%).

Preparation of Ester Solutions Used for Kinetic Studies

Either p-nitrophenyl acetate (0.010 g, 5.5×10^{-5} mol) or p-nitrophenyl caproat (0.020 g, 8.4×10^{-5}) was added to a 10 ml volumetric flask. The flask was filled to the mark with acetonitrile dried by refluxing 3 hr over P_2O_5 followed by distillation. The ester solutions were stored in tightly sealed brown bottles.

Preparation of Polymer Solutions for Kinetic Studies

Into a dry tared 10 ml volumetric flask was weighed 0.1 g of the polymer salt. The flask and contents were dried for three days under vacuum. After drying the flask was reweighed to determine the dry polymer weight. The flasks were then partly filled with glass distilled water and shaken to dissolve the polymer. After standing overnight

to complete the dissolution as well as to allow foam to dissipate, the flasks were filled to the mark. The solutions were shaken and stored in tightly closed brown bottles.

Buffer Solutions

Tris(hydroxymethyl)aminomethane, tris, was used as the buffering agent in the kinetic experiments. Two stock buffer solutions were prepared both 0.1 M in tris and ionic strength. A solution of tris HCl was prepared by adding primary standard tris (12.14 g, 0.1000 mol) and the contents of an acculate 1/10 N hydrochloric acid ampule to a 1000 ml volumetric flask and diluting to the mark with glass distilled water. A solution of tris was prepared by adding primary standard tris (12.14 g, 0.1000 mol) and certified ACS potassium chloride (7.46 g, 0.100 mol) to a 1000 ml volumetric flask and diluting to the mark with glass distilled water. These solutions (both 0.100 M in buffer and ionic strength) were combined to form solutions of the correct pH.

Kinetic Method

To a 4 ml cuvette was added polymer or ethyenediamine stock solution, water and acetonitrile. Amounts of water and acetonitrile were added such that the total volume of polymer stock solution and water was 0.100 ml and the total volume of acetonitrilic ester solution (added later) and acetonitrile was 0.050 ml. Buffer solution (3.00 Ml, 0.100 M tris, I=0.100 M) was pipetted into the cuvette. The pH of the solution was recorded at room temperature, 25±2°C. The

cuvette was placed in a spectrophotometer and equilibrated to 25.0±0.3°C. The ester solution was placed on a glass rod with a flattened tip which was used to add the ester and stir the solution.[14] The rate of increase in p-nitrophenoxide anion concentration was observed at 400 nm until no change in absorbance, A, was observable to determine A_∞. The observed rate constants were calculated from the values of $A_\infty - A_t$ and 6 (time) using a least squares routine of a TI-58 programable calculator. Only the initial portion of the reaction was used to determine the rate constants due to p-nitrophenol inhibition. The rate constants were then corrected for the background rate by subtraction of the value of the background rate constant at the appropriate pH. These corrected pseudo first order rates were then divided by concentration of CH_2CH_2N units, nucleophile concentration or protonated nucleophile concentration (PEI-D-NH_2-HCl) to determine k_{GB}, k_2 or k_2/α, respectively. In those cases where the effect of p-nitrophenol was to be observed, the p-nitrophenol in acetonitrile was added before the buffer, but the total acetonitrile content remained 0.050 ml or 1.6% (Tables IV-5 through IV-12).

Background Rate

The rate of p-nitrophenyl acetate esterolysis without polymer was measured at 7 pH values in the region of interest. The background rate constants (k_{BG}) were found to fit Equation IV-6 (Figure IV-2). The value of k_b[43] (7.0×10^{-2} M^{-1} min^{-1}), k_{OH}[44] (5.70×10^2 M^{-1} min^{-1}) and k_w[43]

Equation IV-6

$$k_{BG} = \frac{k_b[\text{tris}]_{\text{Total}}}{1 + 10^{8.08-pH}} + k_{OH}(10^{14-pH}) + k_w[H_2O]$$

(6×10^{-2} M^{-1} min^{-1}) were available from the literature. The values from this equation were then used for the background rate.

Pyridine Ionization in PEI-D-Pyr-HCl

The pyridine in PEI-D-Pyr-HCl exhibits a pH dependent absorbance at 259 nm. In 0.1 N hydrochloric acid solution the extinction coefficient based on 2 measurements was (5800±400). In 0.1 M tris buffer solution at pH's 6.71, 7.28, and 7.75 the extinction coefficients were found to be 3020, 3030, and 2970, respectively. These values indicate that the state of ionization of pyridine is not changing over pH 6.71 under these conditions.

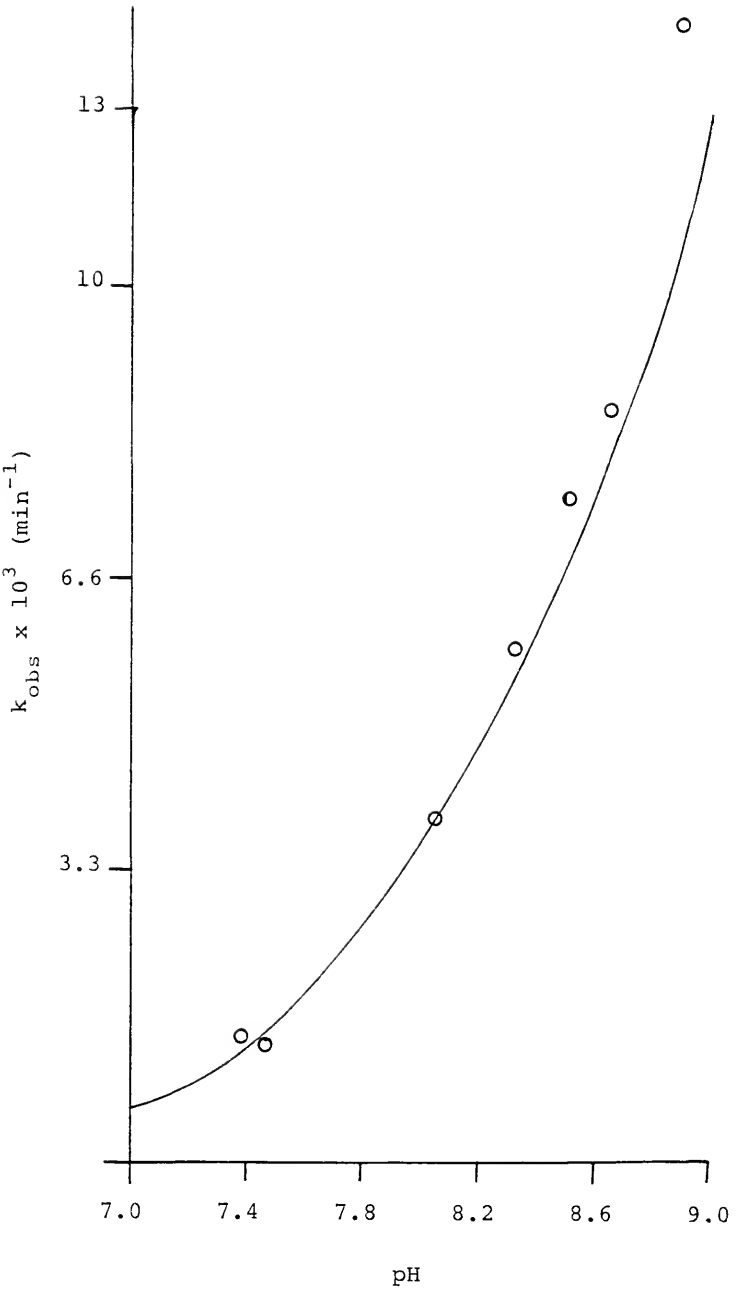


Figure IV-2. Plot of background PNPA esterolysis rate. The line was calculated from Equation IV-6.

TABLE IV-5

ESTEROLYSIS RATES FOR PEI-D-NH_2-HCl

pH	α	k_2 $M^{-1}min^{-1}$	[PEI-D-NH_2-HCl][a]	[PNP][b]	[PNPA][c]	[PNPC][c]	% Reaction Followed
6.94	.54	41	6.8	-	2.0	-	32
7.38	.59	84	13	-	9.5	-	8.2
7.44	.59	88	6.8	-	9.8	-	6.7
7.68	.62	150	6.8	-	2.0	-	39
8.28	.71	430	6.8	-	3.0	-	28
8.47	.72	460	6.7	-	9.8	-	17
8.48	.75	440	13	-	8.7	-	30
8.48	.75	490	13	-	4.4	-	32
8.49	.75	480	13	-	9.5	-	31
8.49	.75	490	6.8	-	1.8	-	30
8.55	.76	500	6.8	-	4.5	-	18
8.52	.75	450	6.8	-	8.9	-	16
8.80	.79	750	6.8	-	2.0	-	15
8.48	.75	460	6.8	-	1.8	-	28
8.48	.75	400	6.8	2.4	1.8	-	31
8.66	.77	530	6.8	4.8	1.8	-	30
6.96	.52	32	6.8	7.2	2.0	-	11
7.68	.61	130	6.8	7.3	2.0	-	11
8.30	.72	320	6.8	7.3	2.0	-	23
8.80	.80	780	6.8	7.3	2.0	-	16
7.00	.55	32	6.8	7.3	2.0	-	80
6.85	.54	25	6.8	9.5	2.0	-	80
7.38	.59	68	6.8	15	2.0	-	80
8.32	.72	250	6.8	15	2.0	-	74
8.82	.80	440	6.8	15	2.0	-	72
8.82	.80	490	2.8	15	2.0	-	77
7.00	.55	710	6.9	-	-	2.6	34
7.39	.59	1600	6.9	-	-	2.6	31
7.53	.50	2200	6.9	-	-	2.6	48
7.70	.62	3300	6.9	-	-	2.6	46

a Primary amine concentration based on 2% primary amine content in PEI-D-NH_2-HCl (x 10^4).

b Initial p-nitrophenol concentration (x 10^5).

c Ester concentration (x 10^5).

TABLE IV-6

ESTEROLYSIS RATES FOR PEI-D-Ip-HCl

pH	k_{GB} $M^{-1}min^{-1}$	[PEI-D-Ip-HCl][a]	[PNPA][b]	% Reaction Followed
7.40	.59	2.7	2.0	36
7.70	.96	2.7	2.0	20
8.34	2.6	2.7	2.0	7
8.82	5.2	2.7	2.0	27

a Concentration of CH_2CH_2N units (x 10^3).
b Ester concentration (x 10^5).

TABLE IV-7

ESTEROLYSIS RATES FOR PEI-D-Im-HCl

pH	k_2 $M^{-1}min^{-1}$	[PEI-D-Im-HCl][a]	[PNPA][b]	% Reaction Followed
6.92	37	8.9	2.0	20
6.97	35	8.9	2.0	81
7.36	65	8.9	2.0	93
7.65	96	8.9	2.0	15
7.98	160	8.9	2.0	93
8.30	290	8.9	2.0	12
8.58	290	8.9	2.0	93
8.81	400	8.9	2.0	19
8.81	380	8.9	2.0	97

a Concentration of imidazole based on 28% imidazole (x 10^3).
b Ester concentration (x 10^5).

TABLE IV-8

ESTEROLYSIS RATES FOR PEI-D-Im-Ip-HCl

pH	k_2 $M^{-1}min^{-1}$	[PEI-D-Im-Ip-HCl][a]	[PNPA][b]	% Reaction Followed
6.90	27	7.8	2.0	93
7.32	43	7.8	2.0	99
7.96	93	7.8	2.0	66
8.59	180	7.8	2.0	67
8.80	230	7.8	2.0	74

a Concentration of imidazole based on 28% imidazole (x 10^3).
b Ester concentration (x 10^5).

TABLE IV-9

ESTEROLYSIS RATES FOR PEI-D-Im-Ip^2-HCl

pH	k_2 $M^{-1}min^{-1}$	[PEI-D-Im-Ip^2-HCl][a]	[PNPA][b]	% Reaction Followed
6.72	19	1.3	2.0	94
7.58	46	1.3	2.0	81
8.25	98	1.3	2.0	96
8.54	140	1.3	2.0	95
8.75	170	1.3	2.0	83

a Concentration of imidazole based on 28% imidazole (x 10^3).
b Ester concentration (x 10^5).

TABLE IV-10

ESTEROLYSIS RATES FOR PEI-D-APyr-HCl

pH	k_2 $M^{-1}min^{-1}$	[PEI-D-APyr-HCl][a]	[PNPA][b]	% Reaction Followed
7.00	14	7.2	2.0	70
7.26	24	13	8.8	21
7.28	27	8.9	9.0	17
7.32	31	4.5	9.1	12
7.34	27	17	8.7	24
7.36	28	8.9	9.0	15
7.36	29	4.5	9.1	7
7.38	29	13	8.8	19
7.40	43	7.2	2.0	82
8.02	96	7.2	2.0	76
8.42	180	17	8.9	15
8.45	130	13.1	8.8	13

a Concentration of 2-aminopyridine based on 28% 2-aminopyridine in PEI-D-APyr-HCl.

b Ester concentration (x 10^5).

TABLE IV-11

ESTEROLYSIS RATES FOR PEI-D-Pyr-HCl

pH	k_2 $M^{-1}min^{-1}$	[PEI-D-Pyr-HCl][a]	[PNPA][b]	% Reaction Followed
6.83	11	9.0	2.0	40
7.22	22	14	8.8	20
7.26	22	9.2	9.0	14
7.32	24	4.7	8.8	15
7.35	26	9.2	9.0	9
7.60	52	9.2	2.0	24
7.94	74	14	8.8	17
7.96	81	9.2	9.0	14
7.98	66	9.2	9.0	37
8.02	87	4.7	9.1	17
8.38	160	14	8.8	17
8.44	170	9.2	9.0	21
8.48	170	4.7	9.1	27
8.78	280	9.1	2.0	26

a Concentration of pyridine based on 17% pyridine in PEI-D-Pyr-HCl.

b Ester concentration (x 10^5).

TABLE IV-12

ESTEROLYSIS RATES FOR ETHYLENEDIAMINE · HCl

pH	k_2 $M^{-1}min^{-1}$[a]	$[H_2NCH_2CH_2NH_2 \cdot HCl]$	[PNPA][c]	[PNP][d]	% Reaction Followed
6.65	5.99	7.86	2.0	0	31
6.66	5.75	7.86	2.0	3.56	47
6.66	5.55	7.86	2.0	7.13	46
6.66	5.15	7.86	2.0	11.8	36

a Literature value 5.6 M^{-1} min^{-1} (no added PNP).[45]

b Concentration of mono hydrochloride (x 10^3) based on pK_{a_2}=7.14.[45]

c Ester concentration (x 10^5).

d Initial p-nitrophenol concentration (x 10^4).

APPENDIX

Definition of pK_a in Polymer Systems

The multiplicity of pK_a definitions for polymer systems in the literature creates some ambiguity as to the meaning of polymer pK_a. Kunitake and Shinkai[46] have defined three different polymer pK_a values.

$$pK_a = pH + n'Log\frac{1-\alpha}{\alpha}$$

$$pK_{a,app} = pH + Log\frac{1-\alpha}{\alpha}$$

$$pK_{a,int} = pK_{a,app} - \frac{0.43\Delta G_{el}}{RT}$$

The following passages quoted from Morawetz[47] helps clarify the issue:

> With a polymer carrying a large number of ionizable groups, it is obviously impracticable to specify the successive ionization constants. Instead of this, we define the apparent ionization constant K_{app} of an average ionizable group carried by the polyion in the usual manner by
>
> $$(H^+)\alpha_1/(1-\alpha_1) = K_{app}$$
>
> where K_{app} will, of course, vary with the degree of ionization since the charged polymer will interact with the hydrogen ions. With polymeric acids the polyanion will attract the hydrogen ions and $dK_{app}/d\alpha_1 < 0$; with polymeric bases, on the other hand, the hydrogen ions will be repelled by the polycation and the acid strength of the polymer will increase with its charge density. If the required electrostatic free energy for the removal of an

equivalent of protons at a given degree of ionization is $\Delta G^{i}_{el}(\alpha_1)$ then

$$pK_{app} = pK^{\circ} - 0.43\Delta G^{i}_{el}(\alpha_1)/RT$$

where K° is characteristic of the ionizing group under conditions where electrostatic interactions with other ionizing groups are absent.

The investigation of base strength-reactivity effects requires an accurate measure of basicity. The base strength of a poly-functional base varies with α. Therefore the value of pK_a used in the correlation of rate with reactivity must take into account the effect of α on basicity. The intrinsic pK_a (pK_a, $pK_{a,int}$, or pK°) is devoid of α dependence. The value of $pK_{a,app}$, on the other hand, is a function of α and is determined readily from pH and α data. The use of $pK_{a,app}$ obviates the necessity of determining the value of ΔG_{el} as would be required if intrinsic pK_a's were used (see above). Therefore $pK_{a,app}$ is the parameter of choice for the base strength-reactivity correlation.

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BIOGRAPHICAL SKETCH

Curtis Stanley Lege was born on Devember 27, 1946, in Princeton, Indiana. His family moved to Orlando, Florida, in 1947. He graduated from Maynard Evans High School in 1964. Mr. Lege enlisted in the United States Air Force and received an Honorable Discharge in December 1969. Shortly after return to civilian life he married the former Joe Ann Purdue of Defuniak Springs, Florida, on February 21, 1970. He received an Associate of Arts degree from Valencia Community College in June of 1972. Later he received his Bachelor of Science degree from the University of West Florida in June 1974. He entered the doctoral program at the University of Florida in the fall of 1974 where he pursued a degree in organic chemistry. During this time the Lege family's first child, Spring Ann, was born on July 15, 1979. Upon graduation, he will begin work as a Research Chemist at the Westvaco Corporation's Charleston Research Center.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

James A. Deyrup, Chairman
Professor of Chemistry

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree Doctor of Philosophy.

Merle A. Battiste
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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree Doctor of Philosophy.

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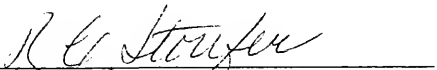
Professor of Biochemistry

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.



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This dissertation was submitted to the Graduate Faculty of the Department of Chemistry in the College of Liberal Arts and Sciences and to the Graduate Council, and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

December 1979

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